Status Report on
Pharmacological Research in India,
1994-1998

Editor

C. ADITHAN, M.D., Ph.D.,
D.N.B., FIMSA., FIPS.

INDIAN NATIONAL SCIENCE ACADEMY
Bahadur Shah Zafar Marg, New Delhi-110 002.

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FOREWORD

Research in Pharmacology forms a major segment of biomedical studies in India. For many years, the largest number of projects funded by the Basic Medical Sciences Division of Indian Council of Medical Research have been in Pharmacology.

The Academy sent a full fledged delegation to IUPHAR Council Assembly for the first time at the Tokyo Congress in 1981. We realised the rather low international visibility of Indian contribution. This was largely due to most of the work being published in Indian journals, majority of which were not accessible in international data bases. Academy's national committee for IUPHAR, therefore, decided to prepare a status report or current research activities in Pharmacology in India. The first report covering the period upto 1983 was published in 1984. I had the privilege of presenting it at the IUPHAR General Assembly in London the same year. It was very well received by the Assembly and appreciated by the IUPHAR officials. I am happy that the publication of the status reports has continued to be an important activity of the National Committee since then and three volumes have been published so far.

The present volume covers the work done after the last report and till the middle of 1999. It has certain unique features. Firstly, the Indian Journal of Pharmacology, official publication of Indian Pharmacological Society, will publish the entire report in one of its regular issues. This will make it available to a much larger community of pharmacologists, other research workers and administrators. Indian Journal of Pharmacology has the largest circulation among the journals publishing research work of Indian pharmacologists and indexed in major global data bases. Secondly, the journal has also agreed to host the report on its website (www.ijp-online.com). It should also be available on the website of the academy. Finally, the report includes, for the first time, a review of the work done by Indian pharmacologists in USA and Canada during the last few years. Prof. P.N. Patil graciously undertook this difficult assignment.

I would like to thank Dr. C. Adithan, a member of the National Committee for editing and ensuring timely publishing of this report. I would also like to express my gratitude to Dr. P.K. Seth and Dr. R.C. Srimal who helped me in reviewing the manuscripts. I hope this useful activity will be continued by Academy's future National Committees for IUPHAR.

20.06.2000
Lucknow

B.N. DHAWAN
Chairman

National Committee for IUPHAR
Indian National Science Academy

EDITORIAL

Pharmacological research in India was qualitatively and quantitatively very high in the late 60s and early 70s. Shanti Swarup Bhatnagar prize, the most prestigious national award, was awarded to Dr. U.K. Sheth (1968), Dr. Ranjit Roy Chaudhury (1969) and O.D. Gulati (1971). Later there was a decline in the trend and in the last 15 years it was not awarded to any pharmacologist. An earlier survey of 1996 showed a definite decline in the number of research papers presented in the IPS conferences. There is an urgent need to reverse this trend and in this connection, it is required to know about the current status of pharmacological research activities of various laboratories.

This publication may serve as a single source of information on Pharmacological research works done in India during 1994-1998. The National Committee for IUPHAR carefully selected the contributors of each chapter. The contents of each chapter provide in a concise manner, the research contribution by Indian pharmacologists in their specialty. Most of the research works are described in brief without going into the details, which the readers can obtain either by reading the concerned reference or by contacting the original authors. This publication may help the readers in identifying fellow research workers, which may pave way for future interaction and collaboration.

The salient points of the reviews are: Indigenous drugs continue to attract many research workers; Toxicology is emerging as an important field; the once popular ANS and Cardiovascular research have lost their charm; Clinical Pharmacology needs further impetus; very few research problems related to the National Health priority are undertaken by Indian pharmacologists; very few pharmacologists continue to work in one field and some of the once active centres have ceased to be active. There is a qualitative change in the pharmacological research in recent times in Western countries. Advent of molecular biology, receptor cloning, *in vitro* tissue culture techniques, gene therapy *etc.*, have forced many Western laboratories to switch over their research from classical pharmacology to molecular pharmacology. But in India we continue to work on classical pharmacology. We have to follow new and more efficient molecular and *in vitro* techniques. Otherwise we will end up with re-inventing the wheels.

The sources of information for the write up are Medline search, manual search of journals published in India (which are not covered by Medline), annual reports of government agencies, centres associated with National Health Programmes, reprints sent by respondents (which are very few) to the advertisement published in Indian Journal of Pharmacology. If some publication has not been included, it is not intentional but it is due to non-availability of information from research workers.

I thank all the contributors for their excellent co-operation and pain taking efforts in collecting the information and for presenting it well within the time frame. I also thank Prof.B.N.Dhawan for his foreword and for going through the manuscript. I wish to thank Dr.R.C.Srimal and P.K.Seth for their valuable comments.

The financial assistance provided by the Indian National Science Academy, New Delhi is gratefully acknowledged.

Pondicherry
August 10, 2000.

C.Adithan

PHARMACOLOGY OF AUTACOIDS

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1. Introduction

Autacoids are the substances released from the cells in response to various types of stimulation to elicit normal physiological responses locally. An imbalance in their synthesis, release or in the transduction system contributes significantly to pathological conditions such as inflammation, allergy, hypersensitivity and ischaemia - reperfusion. Nitric oxide (NO) was recognized by Science[1] magazine as the molecule of the year in 1992. It has led to a spurt in the research on NO and oxygen derived free radicals, which is very evident throughout the world and also in India. Research work carried out in India during the 'report period' on NO and reactive oxygen species (ROS) has also been included in this article. This review includes the papers published in this area during 1994-1998 but does not cover abstracts or conference proceedings.

2. Studies on central nervous system

Serotonin (5-HT) plays an important role in the regulation of various cognitive behavioural functions such as sleep, mood, learning, pain, depression and anxiety by binding to its receptors on the cell membranes. Alterations in the 5-HT synthesis, metabolism and in the receptor characteristics leads to various pathological conditions. Chattopadhyay *et al.*[2, 3] have worked on the solubility, absorbance and fluorescence characteristics of the 5-HT$_{1A}$ receptor purified from buffalo brain and its interaction with 5-HT. These studies might help further in understanding the interaction of 5-HT with its receptor.

The mouse 5-HT$_{1F}$ receptor, which is predominantly expressed in the cortex and hippocampus, is encoded by at least three transcripts as is also observed in the rat brain. The mouse 5-HT$_{1F}$ clone shows the coding region to be intron less and an intron splice junction is seen in 5'-untranslated region that is conserved both in the rat and mouse[4].

Intracerebroventricular (*icv*) administration of serotonin antibodies (5-HTabs) in Balb/c mice leads to alteration in the behaviour as well as in the levels of 5-HT, dopamine (DA) and their metabolites in the substantia nigra (SN), nucleus raphe dorsalis (NRD) and caudate putamen. 5-HTabs inhibited tail flick latency but the tremor response to 5-methoxy N,N-dimethyl tryptamine was augmented. 5-HTabs also induced increase in the 5-HT turnover in all the nuclei studied; however, DA turnover was increased only in SN. This study helps in understanding the central neurotransmitter control on behaviour. It might also help in developing a 5-HT deficient model for studying clinical disorders but it may not help in delineating the underlying mechanism[5].

5-HT uptake inhibitor, fluoxetine, is metabolised in the brain by the enzyme flavin monooxygenase, thus this enzyme might be involved in local metabolism and modulation of the pharmacological effects of the psycho-active drugs[6]. It metabolises fluoxetine to its respective N-oxide and S-oxides[7,8]. Extirpation of the adrenal glands *per se* did not alter the responses to noxious stimuli but it increased the anti-nociceptive potency of imipramine in the rat[9]. The anticonvulsant activity of the sepia shell might be due to the elevation of the seizure threshold rather than the prevention of the seizure spread. This action seems to be mediated by the serotonergic transmission[10]. *Panax ginseng* and diazepam both attenuated stress induced elevation in brain and hypothalamus 5-HT level and in the plasma corticosterone levels[11]. Pyrimidine thiols exhibit anticonvulsant activity, which seems to be mediated by enhancing the GABA-ergic activity and opening the chloride channels[12]. Twenty patients of depression treated with imipramine as well as centpropazine did not exhibit significant changes in the blood pressure, heart rate, PR, QRS, Q-Tc intervals[13].

Oil extract of *Celastrus paniculatus* seeds improved the retention ability in rats. NE, dopamine, 5-HT and their metabolites in brain and urine were significantly decreased in the treated group suggesting a decrease in their turnover to improve the learning and memory[14]. The acetone soluble fraction of petroleum ether extract of *Lawsonia inermis* leaves exhibited prominent nootropic activity and modified the 5-HT and NE mediated behaviour, suggesting towards exploring its nootropic principle[15]. Subordination induced decrease in the 5-HT and dopamine contents in the frontal cortex have been observed in the rats[16].

Involvement of nitric oxide and oxygen derived free radicals has been suggested in the neurodegeneration observed in the Parkinson's disease and also in the cerebral ischaemia. Studies carried out in India have also investigated their role in the experimental models of chemical and ischaemia induced lesions. A significant decrease in the phospholipids and membrane fluidity was observed, following 6-hydroxydopamine (6-OHDA) induced bilateral lesions in the striatum. In addition, there was a significant increase in intracellular calcium and malondialdehyde (MDA) levels. However, glutathione content and the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) was attenuated 72 hr after 6-OHDA injection into the striatum[17]. Studies on another neurotoxic substance, methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) were performed in golden hamsters. These animals were resistant to the toxicity observed following both acute and chronic administration of MPTP as indicated by lack of any alteration from normal content of dopamine and its metabolites in the nucleus caudate putamen (NCP), limbic system and SN. Balb/c and C57/BL mice exhibited >50% and 70% depletion in DA following MPTP administration respectively. The content of total monoamine oxidase (MAO) in golden hamster was one third to one sixth of any nuclei or mitochondria of both strains of mice. Differences in the distribution and levels of MAO could be correlated with the MPTP neurotoxicity[18]. NO by reacting with superoxide radicals (O^-_2) generates more toxic and reactive peroxynitrite, reactive nitrogen species, which on decompositions form more toxic and reactive hydroxyl radicals. This pathway has been found to mediate neurodegeneration and neurotoxic action[19]. Rapid restoration of thiol homoeostasis in the brain during reperfusion following cerebral ischaemia helps in the recovery from reperfusion induced injury. It has been observed that glutathione is significantly decreased after the reperfusion for 1 hr following moderate or severe ischaemia for 30 min[20]. Tertiary butyl hydroperoxide caused increase in the lipid peroxidation and decrease in the reduced glutathione content in the striatum, which was prevented by alpha tocopherol and nicotinamide[21].

Inhibition of endothelial nitric oxide synthase in the periphery is primarily known to be associated with hypertension. However, involvement of NO/Arginine/cGMP pathways in the central regulation of blood pressure is not very well understood. Studies on normotensive cats showed the involvement of NO/Arginine/cGMP pathways in the central regulation of blood pressure[22]. Topical application of sodium nitroprusside (SNP) produced a significant hypotension and bradycardia, which was significantly blocked by methylene blue (MB), suggesting towards the possible involvement of NO-cGMP mechanism in the central cardiovascular regulation[23]. An interrelationship between blood pressure level and brain nitric oxide synthase (NOS) activity in hypertensive rats has also been indicated. NOS activity was significantly reduced in the medulla and hypothalamus of coarctation induced experimental model of hypertension in the rat. In the same study anti-hypertensive drugs, captopril, nifedipine, or centhaquin (a centrally acting antihypertensive compound developed at CDRI) normalised blood pressure and the brain NOS activity[24]. Cyclosporin A, a potent immunosuppressive agent, by interfering with calcium /calmodulin, inhibited the brain NOS activity both *in vitro* and *in vivo* in the rat[25].

The permeability of the blood brain barrier (BBB) is altered in several metabolic derangements, infections, poisoning and other pathological conditions. NO mediated modulation of BBB permeability has been demonstrated by Shukla *et al.*[26]. Induction of NOS activity after exposure to lipopolysaccharide contributes to the increase in BBB permeability[27]. Thus the results obtained demonstrated the involvement of NO/Arginine pathway and reactive oxygen species[26-28] in the opening of the BBB.

Ginkgo biloba, a platelet activating factor (PAF) antagonist, potentiates the picrotoxin-induced convulsions, which might be due to the involvement of GABAergic system and chloride channel. While facilitation of the strychnine action suggests towards the modulating action of *Ginkgo biloba* on glycine[29]. *Ginkgo biloba* treatment decreased the protective effect of sodium valproate and carbamazepine against tonic convulsions in mice[30].

3. Studies on peripheral system

Calcium channel blockers (CCBs) significantly reduced the 5-HT induced contractions and completely blocked histamine induced contractions in the rat aortic rings. Similarly histamine and 5-HT induced contractions were also attenuated in aortic rings obtained from DOCA saline hypertensive rats in presence of these blockers[31]. In DOCA saline hypertensive rats chronic treatment with prazosin reduced the pD_2 value for norepinephrine (NE) and abolished the phenylephrine (PE) response in isolated aortic strips. However, chronic treatment with thyroxine and prazosin significantly augmented the pD_2 value for NE but it was attenuated for PE in comparison to the thyroxine treated controls[32]. Furthermore chronic treatment with ethinyl oestradiol for 3 weeks did not alter the pD_2 values of NE in the rat isolated aortic strips, however, pD_2 values of NE in the portal vein preparation was significantly increased[33].

Role of central serotonergic system in various pathologies has been reported. Changes in the serotonergic systems in the central nervous system have been reflected in the platelets as shown by many investigators. A significant low basal platelet 5-HT and cerebrospinal fluid 5-hydroxy indole acetic acid (5-HIAA) concentration in epileptic patients suggests the involvement of 5-HT in epilepsy[34]. Platelet 5-HT_2 receptors have also been found to be associated with severe social phobia[35]. Platelet 5-HT basal level uptake and efflux was not significantly different from the 8 untreated depressive adolescents from age and sex matched controls. However, fluoxetine, a 5-HT uptake inhibitor produced clinical recovery in all the patients and reduced the 5-HT content and uptake as compared to the control and pre-treatment level[36]. Increased sympathetic activity seems to be responsible for an increase in the platelet 5-HT, epinephrine and norepinephrine content in the diabetic patients and also in the streptozotocin treated rats[37]. Increase in the 5-HT levels seems to be associated with toxaemia of pregnancy[38]. In the patients of essential hypertension an increase in the platelet intracellular calcium level seems to be due to a decrease in the Ca^{++} ATPase activity[39].

Central serotonergic system seems to play a critical role in the immunomodulation[40]. Immue-21, a polyherbal product significantly potentiated humoral immunity in rabbits[41]. Ascorbic acid stimulates the humoral immunity by increasing synthesis of IgG, IgA and IgM and it also activates the macrophages[42]. Benzodiazepine-GABA A and B receptors appear to regulate the restrain stress induced modulation in release/activity of leucocyte migration inhibition factor, a lymphokine released from the sensitized lymphocytes[43]. In another study human aqueous placental extract was found to be immunostimulant for humoral and cell mediated immunity in animals as well as in humans[44].

The response to 5-HT was inhibited while that of NE was potentiated in the anococcygeus muscle and vas deferens after 30-min incubation with fluoxetine. Treatment of rats with fluoxetine for seven days suggests towards the depletion of catecholamines[45]. Contractile activity of *Alstonia boonei* (used in West Africa to treat fever and malaria) extract observed in the isolated rat stomach strip and guinea pig ileum was antagonized by methysergide suggesting the role of 5-HT in the biological effect of the extract[46].

Nifedipine offered significant protection against the 5-HT induced increase in the paw oedema and reduced the volume of the fluid and weight of the granulation tissue in the granuloma pouch but verapamil and diltiazem exhibited no protection[47]. Nifedipine, verapamil and diltiazem produced significant and dose dependent anti-inflammatory effect in rat hind paw oedema produced by prostaglandin E_1 (PGE_1) and bradykinin[48], which was better than aspirin. These inhibitors were ineffective against the histamine-induced increase in the paw volume[47,48].

NO donor, 3-morpholinosydnonimine (SIN-1) induced a concentration dependent relaxation in the coronary artery rings precontracted with 30 mM K^+. Pretreatment of the rings with methylene blue, a guanylate cyclase inhibitor, shifted the SIN-1 induced response curve towards right. Glibenclamide, a K^+-channel blocker blocked the pinacidil induced response but it had no effect on SIN-1 induced response[49], suggesting that NO mediated relaxation is not mediated by K^+-$_{ATP}$ channels. On the other hand pinacidil induced vasorelaxation in 30 mM K^+ preconstricted rings was blocked by NOS inhibitor L-NAME, MB and by endothelium denudement. However, levecromakalim (another K^+-$_{ATP}$ channel opener) induced relaxation was potentiated in the absence of basal NO. Papaverine induced relaxation also remain unaltered by basal NO. Thus basal NO deferentially modulates the interaction of pinacidil and levecromakalim with the K^+- (ATP) channels in goat coronary artery through a cGMP-dependent pathway[50]. NO mediated attenuation of the reactive oxygen species (ROS) dependent norepinephrine-induced contraction of the aortic rings has also been reported. The effect was not observed in the endothelium denuded preparations, suggesting that NO interact with ROS in the endothelium during contraction[51]. Similar attenuation in the acetylcholine induced NO release and augmentation in the endogenous ROS generation plays an important role in the modulation of vasoreactivity in the renal hypertensive rats[52]. Thus both NO and ROS considerably influence vascular reactivity in the normotensive as well as in hypertensive rats. Methylene blue attenuated the acetylcholine (ACh) induced relaxation in the endothelial intact monkey aortic strips; therefore it appears that in these strips ACh increases the intracellular cGMP levels. However, ACh induced contractions in the endothelial denuded rings were further augmented[53].

Polymorphonuclear leukocyte (PMNL), the cohabitant of platelets in blood, release several factors that alter platelet response. Following thrombosis circulating PMNLs release more NO and less free radicals[54] and this could be a defence mechanism of the body. A factor was found to be present in the PMNLs cell suspension, which inhibits ADP, arachidonic acid and calcium ionophore induced platelet aggregation[55]. Nonsteroidal anti-inflammatory drug, aspirin when taken along with nifedipine, a calcium channel antagonist and anti-hypertensive drug exhibited a synergistic inhibitory effect on collagen, ADP and epinephrine induced platelet aggregation[56].

Free radical scavengers, PAF-antagonists[57], cyclo-oxygenase inhibitors and NO releasing compound have been found to be effective in preventing paralysis/death due to intravenous collagen and adrenaline injection in mice and rats. These findings suggested that free radicals could be involved in the genesis of thrombosis. Therefore role of free radical generating and scavenging enzymes in this pathological state had been investigated[58,59]. Involvement of free radicals and neutrophils in thrombosis has been suggested[54]. The oxidative burst of PMNLs is primarily meant for the killing of ingested micro-organisms. However, research on these cells has shown that they also play an important role in ischaemia reperfusion, inflammation and thrombosis. PMNLs release both NO and ROS. Investigation on the interaction of NO with superoxide radicals suggested towards the possibility of NO regulating the generation of ROS. NO donors like sodium nitroprusside (SNP) and diethylamine-nitric oxide (DEA-NO) as well as NO precursor, L-arginine inhibited formyl-methionine-leucyl-phenylalanine (FMLP, a chemotactic peptide), arachidonic acid (AA, NADPH-oxidase activator), phorbol ester (PMA, protein kinase C activator) or opsonized zymosan (OZ, a phagocytic ligand) induced luminol-dependent chemiluminescence (LCL), hydroxyl radical generation, oxygen consumption and NADPH-oxidase activity in the PMNLs. Both exogenous and endogenous NO inhibited the NADPH-oxidase activity. Thus superoxide radical generation was inhibited and NO also scavenged the free radicals released/generated from PMNLs[60]. NOS inhibitors and their D-enantiomers attenuated the LCL response, which was not due to non-availability of NO. Therefore, effect of L-arginine analogues, and their D-enantiomers was also investigated on NADPH-oxidase activity and oxygen consumption of the rat PMNLs[61]. It was seen that NO mediated attenuation of LCL response was due to the inhibition of NADPH-oxidase activity and oxygen consumption. Nevertheless, NOS inhibitors and their antipodes inhibited LCL response without inhibiting NADPH-oxidase activity and oxygen consumption. The results obtained thus warrant caution in using NOS inhibitors (arginine analogues) to analyse the role of NO in PMNLs LCL response, as they themselves seem to scavenge free radicals[61].

In an interesting study on the free radical generation from PMNLs in control and in smokers (cigarette, bidi, hookah and mixed products), a significant increase in the superoxide radical generation was observed. Role of PMNLs and free radicals has been suggested in the chronic obstructive pulmonary disease[62]. NO production in the 26 patients of systemic lupus erythematosus was increased in comparison to the control, as evident by an increase in the serum nitrite and citrulline levels[63].

Effect of *Pasteurella multocida* on hydrogen peroxide (H_2O_2) and NO generation from isolated PMNLs was studied in control and in vaccinated buffaloes against haemorrhagic septicemia. Lipopolysaccharide (LPS) of *P. multocida* and live *P. multocida* both potentiated the release of H_2O_2 and NO from the control and the vaccinated animal cells in comparison to the non-activated cells. However, PMNLs isolated from vaccinated animals exhibit a significant increase in H_2O_2 and NO release in comparison to control. Thus results obtained indicate an up regulation in oxidant defence system in presence of an antiphagocytic bacterium, in the PMNLs[64]. Septilin, an Ayurvedic formulation, showed significant protection in *E. coli* induced abdominal sepsis in normal mice and *Staphylococcus* aureus induced sepsis in neutropenic mice. It also stimulated the phagocytic function of neutrophils and reticuloendothelial system in mice. In normal rats, Septilin enhanced anti-sheep RBC agglutination antibody titer by 6 folds and showed significant protection against cyclophosphamide - induced humoral suppression[65]. Involvement of NO in the hypoglycemic activity of tolbutamide is suggested[66].

Calcium modulating agents such as EGTA, nifedipine, TMB-8 and calmodulin antagonist W_7 inhibited the Cisplastin (CP, a potent chemotherapeutic drug) induced tumoricidal activity of peritoneal macrophages by decreasing the release of tumor necrosis factor-α (TNFα) and interleukin-1 (IL-1) in the supernatant[67]. CP and LPS pretreatment of murine peritoneal macrophages showed an enhanced production of NO and a tumoricidal activity against P815 mastocytoma cells. L-Nitro mono methyl arginine (L-NMMA) a specific NOS inhibitor, blocked this response[68]. Protein kinase C (PKC) inhibitor, H_7 and chelerythrine chloride and tyrosine kinase inhibitor genistein and levendustin A, inhibited the CP induced IL-1 and TNFα secretion and tumoricidal activity of macrophages[69], thus suggesting the involvement of PKC and tyrosine kinase in activation of peritoneal macrophages with CP. Expression and secretion of Oncostatin M, a novel cytokine that regulates growth was increased following CP stimulation of macrophages. It might be responsible for the cytotoxic and cytostatic activity of CP[70]. CP in addition increases the intracellular NFκB content and its translocation, which involves serine/threonine phosphatases, protein tyrosine phosphatases, genistein sensitive protein tyrosine kinase activities[71]. Antioxidants such as glutathione, N-2 mercapto propyl glycine, vitamin E and C exhibit protective effect against CP induced emesis in dogs and rats[72,73]. Certain antineoplastic drugs apart from their tumoricidal activity are also capable of acting as immunostimulants. Supernatant collected from cells treated with cytosine arabinoside (CA), vincristine sulfate (VS), cyclophosphamide (CS), mitomycin C (MMC), hydroxyl urea (HU) or LPS enhanced production ofTNFα, IL-1, NO and lysozyme, thus indicating towards the macrophage activating activity of these agents[74].

A traditional silver preparation used in the Indian systems of medicine exhibits moderate analgesic effects suggesting possibly the involvement of opioidergic mechanisms[75]. Activity of the glycolytic enzymes was significantly decreased while an increase in the gluconeogenic enzymes was observed and Serankottai Nei (a Siddha drug) prevented these changes in the arthritic rats[76]. The icv administration of Substance P, significantly accentuated formaldehyde induced oedema volume along with proalgesic response thus, suggesting the pro-inflammatory effect of substance P[77].

Indole, triazine, benzopyran derivatives and substituted quinazolines were evaluated for anti-inflammatory activity in rats[78-84]. A crude extract from the roots of *Gmelina asiatica* exhibited anti-inflammatory activity against carrageenan induced rat paw oedema and cotton pellet granuloma models in the rat. Antiproliferative, anti-oxidant and lysosomal membrane stabilisation activity of the drug[85] possibly mediated the inhibitory effect. 4',5,6-trihydroxy-3', 7-dimethoxy flavone-from *Vicoa Indica* DC showed consistent anti-inflammatory and analgesic activity in various models[86]. Methanolic and aqueous extract of *Curculigo orchioides* exhibited significant anti-inflammatory activity in comparison to ibuprofen and indomethacin[87]. Effect of triterpenes from *Crataeva nurvala* stem bark has been investigated on adjuvant induced arthritis in the rats[88]. The water-soluble extract of the alcoholic extract of *A. indica* leaves exerted significant anti-inflammatory activity in cotton pellet granuloma assay in the rat[89]. Synthetic analogues of liposomes prepared from non-ionic surfactants known as niosomes have been used to prepare the trapped diclofenac sodium. Formulations of diclofenac prepared by 1:1 combination ofTween 85 and poloxamer F-108 exhibited better and consistent anti-inflammatory activity for more than 72 hr after administration of a single dose[90].

Combination of the non-steroidal anti-inflammatory drugs diclofenac, pitofenone and fenpiverinium might have antispasmodic activity as they inhibited the ACh induced contractions in the rat colon[91]. Effect of ATP, adenosine or caffeine was investigated on Brewer's yeast induced peripheral inflammation and nociception after their *icv* administration. The results indicated that central purinergic system exerted anti-inflammatory effect[92]. Further study showed that centrally administered $PGF_{2\alpha}$ exerts anti-inflammatory while PGE_2 exerts pronociceptive effects on Brewer's yeast induced peripheral inflammation and pain[93].

Riboflavin supplementation showed increased activity of the glutathione redox cycle enzymes and glutathione content in the selinite induced cataractous rat lenses[94]. *Ocimum sanctum* exhibits anti-cataract activity in different experimental models, which might be due to the free radical scavenging[95].

Involvement of both H_1 and H_2 receptors in the production of histamine aerosol induced bronchospasm in the guinea pigs is observed[96]. Sublingual nifedipine exhibited clinical improvement in the moderately symptomatic, non-smoking allergic bronchial asthma patients[97]. Ethanolic extract of the leaves of *Vitex negundo Linn* inhibited the immunologically induced degranulation of the mast cells. It also inhibited the oedema during active paw anaphylaxis in mice. It appears that this extract has the ability to alter calcium fluxes from the extracellular medium and in the membrane[98]. Furosemide significantly attenuated compound 48/80 and egg albumin induced contractions in the guinea pig tracheal rings by either causing local release of PGE_2 or by affecting the mediator release from mast cells[99]. Fluticasone alone and in various formulations effectively reduced histamine induced wheal and flare[100]. Ethanolic extract of *Vitex negundo Linn* inhibited the 48/80 and antigen induced contractions in the guinea pig trachea. The results suggest that it interferes with histamine and arachidonic acid metabolite mediated responses[101]. 5-Methyl benzoxazoline-2-one inhibited the contractile response to histamine, 5-HT, acetylcholine and barium chloride in the guinea pig ileum but not to the same extent suggesting a non-specific antagonism of the papaverine or sympathomimetic agents[102]. Coleonol, an adenylate cyclase activator, was shown to exert significant antipassive cutaneous anaphylaxis and mast cell stabilising activity[103]. CDRI compound, 88/765 (4-amino-6-methyl thio-1-(2', 2'-diethoxyethyl)-1H-pyrazolo [3,4-d]pyrimidine) inhibited the passive cutaneous anaphylaxis in rat and mice and mast cell degranulation of normal and passively sensitised rats induced by 48/80 or egg albumin, the effect was comparable to sodium cromoglycate104. Bleomycin, the glycopeptide antibiotics used in the cancer therapy, induced injury was protected by curcumin due to the restoration of the antioxidant status in the rats[105]. Superoxide dismutase alone or in combination with glutathione reduces the clastogenic action of bleomycin[106]. Five analogues of Ala-Asp-Ser-Asp-Gly-Lys (ADSDK) with modification at position 1,2 and 6 have been tested for anti-allergic activity, one of the peptides (compound 94/335) has shown anti-allergic activity in various experimental models of allergy even by the oral route[107,108]. Development of mast cells from bone marrow precursor cells was performed in cultures using Concanavalin A stimulated splenocyte supernatant[109].

Prolonged treatment with oestrogen or progesterone led to a down regulation of histamine H_1 and H_2 receptors in the rat and guinea pig. The possible mechanism appears to be oestrogen and progesterone induced elevation of the histamine levels in the blood[110].

Diflunisal, a salicylic acid derivative produced significant changes in the neural tube development of chick embryo. PGE_1 and $PGF_{2\alpha}$ treatment potentiated the effect of diflunisal. It appears that diflunisal induced changes might be mediated at the cellular level[111]. L-NAME pretreatment led to a failure of implantation when administered at 2.5 mg/kg per uterine horn on day 3 of pregnancy. L-NAME mediated effect was reversed by SNP pretreatment, suggesting a role of NO in implantation[112].

Cauvery 100, an Ayurvedic formulation exhibited a potent anti-ulcer effect against indomethacin induced ulcers in the rat[113]. Effect of verapamil, nifedipine and diltiazem was evaluated on experimental models of ulcers in the rat[114,115]. Both verapamil and nifedipine offered protection but diltiazem exacerbated the cysteamine-induced ulcers[115]. Sulpiride, a D_2 receptor antagonist increased the total carbohydrate content and decreased the protein contents and exhibited anti-ulcer activity[116]. An herbal drug formulation, UL-409, displayed cytoprotective action against ethanol induced ulcers in the rat. Alterations in the lipid peroxides and antioxidants status were reversed by UL-409[117,118]. Cholinergic involvement and a partial role of NO in the gastrokinetic effects of exepanol in mice and guinea pig ileum have been demonstrated[119]. It has been recently reported that ulcers in the rat are closely associated with augmented free radical generation[120]. Poly I: Poly C, an inhibitor of interferon inducer was found to inhibit the peptic ulcers in the rat[121]. Endoscopic evaluations of osteoarthritis patients for gastrointestinal problems after treatment with ketorolac and diclofenac sodium suggested that short-term analgesic use of ketorolac is safer[122]. Ketorolac also provides progressively increasing and long lasting pain relief in comparison to nefopam in immediate post-operative period following upper abdominal surgery[123]. Ranitidine, famotidine and omeprazole, potent anti-ulcer drugs, have no significant effect on the hepatic functions and lipid profile when used for a period of 12 weeks in the peptic disease patients[124]. Central adenosine A1 via noradrenergic receptors and GABA-B receptors independently inhibit gastric acid secretion[125].

4. Conclusion

5-HT is involved in the pathophysiology of depression, anxiety, hypertension etc. All the physiological and pathophysiological effects of 5-HT are due to its interaction with various distinct membrane receptors. Thus basic studies on the 5-HT interaction with the receptor and behavioural changes following administration of monoclonal antibodies might help us in understanding the interaction at the receptor level and its involvement in the modulation of the behaviour. In addition, studies on the metabolism of drugs which modulate the concentration and availability of 5-HT in the brain is also quite useful as the variability in the effect of drugs could be partly explained on this basis also. Studies on the herbal drugs have shown that some of these drugs/active components exert their effect by modulating the 5-HT levels in the brain or periphery. Interestingly changes in the central serotonergic system have been reflected in the platelets of the patients of social phobia and in epilepsy patients in comparison to the controls. In the diabetic patients and in the toxemia associated with pregnancy, levels of 5-HT were elevated in the platelets. 5-HT induced rat aortic ring contractions were blocked by the calcium channel antagonists and nifedipine was found to block the 5-HT induced increase in the paw volume thus suggesting that calcium is involved in the 5-HT induced responses.

Studies on NO and free radicals have shown the importance of these mediators in several physiological and pathological conditions. Role of NO in central regulation of blood pressure in both normotensive and hypertensive animals has been observed. In the experimental models of Parkinson's disease and in the cerebral ischaemia involvement of free radicals has been observed. In addition, BBB permeability in the infectious conditions appears to be increased by NO, while in the normal situation it does not seem to play an important role.

NO mediated vasorelaxation is not mediated by the K^{+}-$_{ATP}$ channels. However, vasorelaxant response of K^{+}-$_{ATP}$ channel opener, levecromakalim was potentiated in the absence of basal NO. NE induced vasoconstriction seems to be modulated by the endothelial NO and ROS in the rat aortic rings. In addition ACh induced NO release is attenuated while NE induced ROS generation was potentiated in the rings obtained from hypertensive rats. Methylene blue, a guanylate cyclase inhibitor prevented the ACh induced relaxation in the monkey aortic strips but augmented the ACh induced contraction in the denuded strips.

Studies on PMNLs indicate that NOS inhibitor L-NAME scavenges free radicals. NO also seems to modulate the free radical generation from PMNLs by scavenging as well as by inhibiting the generation of free radicals. In addition, work on NO in relation to Leishmania has also been carried out in India[125-127].

Cisplastin was found to induce the microbicidal activity by inducing the release of various inflammatory mediators including NO. During the report period various natural and synthetic compounds were found to exhibit, anti-inflammatory, anti-ulcer and antiallergic activities.

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REFERENCES

1. Culotta E, Kashland Jr. DE. NO news is good news. *Science* 1992;**258**:1861-5.

2. Chattopadhyay A, Rukmani R, Mukherjee S. Photosynthesis of neurotransmitter: ionization and spectroscopic properties of serotonin. *Biophys J* 1996;**71**:1952-60.

3. Chattopadhyay A, Harikumar KG. Dependence of critical micelle concentration of a zwitterionic detergent on ionic strength: implications in receptor solubilization. *FEBS Lett* 1996;**391**:199-202.

4. Guptan P, Dhingra A, Panicker MM. Multiple transcripts encode the 5-HT$_{1F}$ receptor in the rodent brain. *Neuroreport* 1998;**8**:3317-21.

5. Mohanty S, Steinbusch HW, Ganguly DK, Mohankumar KP. Behavioural and neurochemical alteration following intracerebroventricular administration of anti-serotonin antibodies in adult Balb/c mice. *J Chem Neuroanat* 1998; **14**:141-9.

6. Bharwe S, Bhagwat SV, Shanker SK, Boyd MR, Ravindranath V. Flavin containing monooxygenase mediated metabolism of psychoactive drugs by human brain microsomes. *Brain Res* 1995;**672**:276-80.

7. Bhagwat SV, Bharwe S, Boyd MR, Ravindranath V. Central metabolism of imipramine and purified flavin containing monooxygenase from human brain. *Neuropsychopharmacol* 1996;**15**:133-42.

8. Bhagwat SV, Bharwe S, Boyd MR, Ravindranath V. Further characterization of rat brain flavin-containing monooxygenase metabolism of imipramine to its N-oxide. *Biochem Pharmacol* 1996;**51**:1469-75.

9. Mandhane SN, Chopde CT, Muthal AV. The effect of adrenal steroids on imipramine analgesia. *Indian J Pharmacol* 1994;**26**:218-221.

10. Srinivas V, Haranath Babu M, Reddy TN, Diwan PV, Reddy PUM. Modulation of neurotransmitters in mice brains by an anti-convulsant principle from cuttle bone. *Indian J Pharmacol* 1997;**29**:296-300.

11. Sur TK, Bhattacharya D. The effect of *Panax ginseng* and diazepam on brain and hypothalamic 5-hydroxytryptamine during stress. *Indian J Pharmacol* 1997;**29**:318-21.

12. Gupta AK, Sanjay HP, Kayath HP, Singh A, Sharma G, Mishra KC. Anti-convulsant activity of pyrimidine thiols. *Indian J Pharmacol* 1994;**26**:29-34.

13. Nargund MP, Gupta KC. Effect of imipramine and centpropazine on electrocardiogram in-patients of major depression. *Indian J Pharmacol* 1994;**26**:309-10.

14. Nalini K, Karanth KS, Rao A, Aroor AR. Effects of *Celastrus paniculatus* on passive avoidance performance and biogenic amine turnover in albino rats. *J Ethnopharmacol* 1995;**47**:101-8.

15. Iyer MR, Pal SC, Kasture VS, Kasture SB. Effect of *Lawsonia inermis* on memory and behaviour mediated *via* monoamine neurotransmitters. *Indian J Pharmacol* 1998;**30**:181-5.

16. Raju TR. Subordination induced decrease in the 5-hydroxy tryptamine and dopamine level in frontal cortex - A study using worker-parasite relationship in rats as a model. *Indian J Physiol Pharmacol* 1996;**40**:213-9.

17. Kumar R, Agarwal AK, Seth PK. Free radicalgenerated neurotoxicity of 6-hydroxydopamine. *J Neurochem* 1995;**64**: 1703-7.

18. Mitra N, Mohankumar KP, Ganguly DK. Resistance of golden hamster to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine relationship with low levels of regional monoamine oxidase. *Br J Neurochem* 1994; **62**:1906-12.

19. Mohankumar KP, Steinbusch HW. Hydroxyl radicals and nitric oxide in neurotoxicity and neuroprotection. *J Chem Neuroanat* 1998;**14**:125-7.

20. Shivakumar BR, Kolluri SVR, Ravindranath V. Glutathione and protein thiol homeostasis in brain during reperfusion after cerebral ischaemia. *J Pharmacol Exp Ther* 1995; **274**: 1167-73.

21. Parihar MS, Manjula Y, Bano S, Hemnani T, Javeri T, Prakash P. Nicotinamide and alpha tocopherol combination partially protects t-butylhydro peroxide-induced neurotoxicity: Implication for neurodegenerative diseases. *Current Sci* 1997;**73**:290-3.

22. Hegde LG, Shukla R, Dikshit M, Srimal RC. A study on the central cardiovascular regulation in the cat by L-Arginine-EDRF/NO-cGMP pathway. *Arch Int Pharmacodyn Ther* 1994;**328**:155-64.

23. Verma S, Raghubir R, Patnaik GK. Nitric oxide modulation of cardiovascular responses from ventral surface of medulla in cat. *Indian J Pharmacol* 1997;**29**:125-8.

24. Hegde LG, Shukla R, Srimal RC, Dikshit M. Alteration in the brain nitric oxide synthase activity in coarctation model of hypertension in rats. *Pharmacol Res* 1997;**36**:109-14.

25. Rao MR, Kanji, VK, Salahudeen MK, Wang C, Shekhar V. Inhibition of rat brain nitric oxide synthase activity by cyclosporin A. *Indian J Exp Biol* 1996;**34**:1265-7.

26. Shukla A, Dikshit M, Srimal RC. Nitric oxide dependent blood-brain barrier permeability alteration in the rat brain. *Experientia* 1996;**52**:136-40.

27. Shukla A, Dikshit M, Srimal RC. Nitric oxide modulates blood-brain barrier permeability during infections with an inactivated bacterium. *Neuroreport* 1995;**6**: 1629-32.

28. Shukla A, Dikshit M, Srimal RC. Status of bio-antioxidants in brain micro vessels of monkey and rat. *Free Rad Res Commun* 1995;**22**:303-8.

29. Manocha A, Pillai KK, Husain SZ. Effect of *Ginkgo biloba* on chemoshock in mice. *Indian J Pharmacol* 1997;**29**:198-200.

30. Manocha A, Pillai KK, Husain ZS. Influence of *Ginkgo biloba* on the effect of anticonvulsants. *Indian J Pharmacol* 1996;**28**:84-7.

31. Bhugra P, Gulati OD. Interaction of calcium channel blockers with histamine and 5-HT in the aorta from normal and diseased rats. *Indian J Physiol Pharmacol* 1996;**40**:120-6.

32. Bhugra P, Gulati OD. Interaction of alpha-adrenoceptor antagonists with various agonists in isolated aorta and ventricle from normal and diseased rats. *Indian J Pharmacol* 1996; **28**:77-83.

33. Bhatt JD, Gulati OD. Effects of chronic treatment with ethinyl estradiol on the sensitivity of isolated vascular preparations to noradrenaline. *Indian J Pharmacol* 1996;**28**:113-5.

34. Shukla R, Vrat S, Nag D, Verma M, Shanker K. Platelet 5-HT uptake, basal 5-HT and cerebrospinal fluid 5-HIAA in epilepsy. *Asia Pac J Pharmacol* 1996;**11**:103-7.

35. Chatterjee S, Sunitha TA, Velayudhan A, Khanna S. An investigation into the psychobiology of social phobia, personality domains and serotonergic function. *Acta Psychiatr Scand* 1997;**95**:544-50.

36. Joshi G, Sitholey P, Gujrati VR, Saxena AK, Gurtu S *et al.* Platelet serotonin kinetics in depressant adolescents. *Biog Amines* 1997;**13**: 599-602.

37. James J, Podayatti PS, Paul T, Paulose CS. Platelet monoamine changes in diabetic patients and streptozotocin induced diabetic rats. *Current Sci* 1997;**72**:137-9.

38. Bhattacharya TK, Debnath PK. Role of serotonin in toxaemia of pregnancy. *Indian J Physiol Pharmacol* 1995;**39**:86-8.

39. Gulati S, Khullar M, Sharma BK, Ganguly NK. Platelet calcium pump activity in essential hypertensives and their first-degree relatives. *Mol Cell Biochem* 1996;**156**:37-42.

40. Kumar D, Tripathi HC, Mishra SK, Tandon SK, Raviprakash V, Mishra SC. Role of central serotonergic system in immunomodulation in rats. *Indian J Pharmacol* 1996;**28**: 102-6.

41. De P, Dasgupta SC, Gomes A. Immunopotentiating and immunophylactic activities of Immue-21, a polyherbal product. *Indian J Pharmacol* 1998;**30**:163-8.

42. Ansari KU, Kasturi N, Tewarson SL, Singh S, Pandey RC. An experimental and clinical evaluation of immunomodulating potential of ascorbic acid. *Indian J Pharmacol* 1998; **30**:94-6.

43. Mediratta PK, Sharma KK. Role of benzodiazepine - GABA receptor complex in stress - induced modulation of leukocyte migration inhibitory factor. *Indian J Pharmacol* 1997;**29**:228-32.

44. Ansari KU, Gupta N, Bapat SK. An experimental and clinical evaluation of immuno-modulating potential of human placental extract. *Indian J Pharmacol* 1994;**26**:130-2.

45. Patil MR. Evidence of catecholamine-depleting action of fluoxetine. *Indian J Physiol Pharmacol* 1994;**38**:169-73.

46. Makinde JM, Taiwo OB. Contractile activity of *Alstonia Boonei* stem bark extract on isolated rat stomach strip and guinea pig ileum. *Indian J Pharmacol* 1996;**28**:110-12.

47. Aditya GN, Chattopadhyay S, Mandal S, Roy RK, Lahiri, HL, Maitra SK. Preliminary study on anti-inflammatory effect of calcium channel blockers in albino rats. *Indian J Pharmacol* 1997;**29**:132-4.

48. Aditya GN, Chattopadhyay S, Mandal S, Roy RK, Lahiri, HL, Maitra SK. Possible mechanism of anti-inflammatory activity of calcium channel blockers. *Indian J Pharmacol* 1997; **29**:325-6.

49. Deka DK, Raviprakash V, Mishra SK. K^+-$_{ATP}$ channels do not mediate vasodilation by 3-morpholinosydnonimine in goat coronary artery. *Eur J Pharmacol* 1997;**340**:157-64.

50. Deka DK, Raviprakash V, Mishra SK. Basal NO release differentially modulates vasodilations by pinacidil and levecromakalim in coronary artery. *Eur J Pharmacol* 1998; **348**:11-23.

51. Srivastava P, Hegde LG, Patnaik GK, Dikshit M. Role of endothelial derived reactive oxygen species and nitric oxide in norepinephrine-induced rat aortic ring contractions. *Pharmacol Res* 1998;**38**:265-74.

52. Hegde LG, Srivastava P, Kumari R, Dikshit M. Alterations in the vasoreactivity of hypertensive rat aortic rings: Role of nitric oxide and superoxide radicals. *Clin Exp Hypertens* 1998;**20**:885-902.

53. Savithiri S, Kuruvilla A. Effect of methylene blue on endothelium - dependent relaxation by acetylcholine in monkey aortic strips. *Indian J Pharmacol* 1997;**29**:429-32.

54. Seth P, Kumari R, Dikshit M. Alteration in the free radical

generation and nitric oxide release from rat polymorphonuclear leukocytes following thrombosis. *Thrombosis Res* 1997;**87**:279-88.

55. Kumari R, Singh MP, Seth P, Dikshit M. Inhibition of platelet aggregation by a factor present in the rat peripheral neutrophil supernatant. *Thrombosis Res* 1998;**91**:75-82.

56. Desai AA, Nayak VK, Desai NK, Gupta KC. Effect of nifedipine and aspirin on platelet aggregation: A study in hypertensive patients and normal volunteers. *Indian J Pharmacol* 1995;**27**:167-70.

57. Seth P, Kumari R, Dikshit M, Srimal RC: Effect of PAF-antagonists in models of thrombosis. *Thromb Res* 1994; **76**: 503-12.

58. Kumari R, Seth P, Dikshit M, Srimal RC. Alterations in the bio-antioxidants following thrombosis. *Free Radical Biol Med* 1994;**17**:481-4.

59. Kumari R, Seth P, Dikshit M. Involvement of bio-antioxidants in thrombosis in mice. *Redox Report* 1995;7:191-5.

60. Seth P, Kumari R, Dikshit M, Srimal RC. Modulation of rat polymorphonuclear leukocyte response by nitric oxide and arginine. *Blood* 1994;**84**:2741-8.

61. Dikshit M, Chari S, Seth P, Kumari R. Effect of nitric oxide synthase inhibitors and their antipodes on rat polymorphonuclear leukocyte luminol dependent chemiluminescence response. *Br J Pharmacol* 1996;**119**:578-82.

62. Sharma RN, Deva C, Behera D, Kanduja KL. Reactive oxygen species formation in peripheral blood neutrophils in different types of smokers. *Indian J Med Res* 1997;**106**:475-80.

63. Wanchu A, Khullar M, Deodhar SO, Bammbery P, Sud A. Nitric oxide synthesis is increased in patients with systemicosus. *Rheumatol Int* 1998;**18**:41-3.

64. Roy SC, More T, Pati US, Srivastava SK. Effect of Pasteurella multocida vaccination on buffalo polymorphonuclear hydrogen peroxide and nitric oxide. *Vet Immunol Immunopathol* 1996;**51**:173-8.

65. Rao CS, Raju C, Gopumadhavan BL, Kulkarni RD, Mitra SK. Immunotherapeutic modification by an Ayurvedic formulation Septilin. *Indian J Exp Biol* 1994;**32**:553-8.

66. Satyanarayana S. Involvement of nitric oxide in hypoglycemic activity of tolbutamide. *Indian J Physiol Pharmacol* 1998;**42**:551-4.

67. Sodhi A, Kumar R. Role of calcium and calmodulin in the activation of murine peritoneal macrophages with cisplastin. *Int J Immunopharmacol* 1994;**16**:1003-10.

68. Sodhi A, Kumar R. Production of nitric oxide and its regulation in murine peritoneal macrophages treated with cisplastin and lipopolysaccharide. *Int J Immunopathol Pharmacol* 1994;7:65-77.

69. Kumar R, Sodhi A. Activation of murine macrophages with cisplatin and lipopolysaccharide. Involvement of protein kinase C and tyrosine kinase. *J Clin Biochem Nutr* 1994;**17**: 55-64.

70. Sodhi A, Shishodia S, Srivastava A. Cisplatin stimulated bone marrow derived macrophages secrete oncostatin M. *Immunol Cell Biol* 1997;**75**:492-6.

71. Sodhi A, Singh RAK. Mechanism of NF-κB translocation in macrophages treated *in vitro* with cisplatin. *Immunol Lett* 1998;**63**:9-17.

72. Gupta YK, Sharma SS. Antiemetic activity of anti-oxidants against cisplatin induced emesis in dogs. *Environ Toxicol Pharmacol* 1996;**1**:179-84.

73. Sharma SS, Gupta YK. Effects of antioxidants on the cisplatin induced gastric emptying in rats. *Environ Toxicol Pharmacol* 1997;**3**:41-6.

74. Pai K, Srivastava A, Kumar R, Khetrapal S, Sarmah B, Gupta P, Sodhi A. Activation of P388D1 macrophage cell line by chemotherapeutic drugs. *Life Sci* 1997;**60**:1239-48.

75. Khanna AT, Sivraman R, Vohra SB. Analgesic activity of silver preparations used in Indian systems of medicine. *Indian J Pharmacol* 1997;**29**:393-8.

76. Vijayalakshmi T, Narayanan PJ, Sachidanandam P. Changes in glucose metabolizing enzymes in adjuvant arthritis and its treatment with a Siddha drug: *Serankottai Nei*. *Indian J Pharmacol* 1998;**30**:89-93.

77. Dumka VK, Tandan SK, Tripathi HC, Raviprakash V. Pro-inflammatory and pro-algesic effect of icv administered substance P in rats. *Indian J Pharmacol* 1996;**28**:29-31.

78. Verma M, Tripathi M, Saxena AK, Shanker K. Anti-inflammatory activity of novel indole derivatives. *Eur J Med Chem* 1994;**29**:941-6.

79. Saxena S, Verma M, Saxena AK, Shanker K. Triazines as anti-inflammatory agents. *Arzneim Forsch Drug Res* 1994; **44**:766-9.

80. Bhalla M, Hitkari A, Gujrati VR, Bhalla TN, Shanker K. Benzopyran -2-one derivatives: anti-inflammatory, analgesic and anti-proteolytic agents. *Eur J Med Chem* 1994;**29**: 713-7.

81. Bhalla M, Srivastava NK, Bhalla TN, Shanker K. Synthesis of 1,3,4-thiadiazole [3,2-a]-S triazine 5,7 dithione derivatives and their pharmacological evaluation. *Boll Chim*

Farm 1994;**133**:521-6.

82. Bhalla M, Srivastava NK, Bhalla TN, Shanker K. Synthesis and pharmacological evaluation of 1,2,4-triazine and its congeners. *Boll Chim Farm* 1995;**134**:9-15.

83. Hitkari A, Bhalla M, Saxena AK, Verma M, Gupta MB, Shanker K. Substituted quinazolines and their anti-inflammatory activity. *Boll Chim Farm* 1995;**134**:609-15.

84. Mishra P, Gupta PN, Shanker AK, Shukla R, Srimal RC. Anti-inflammatory and diuretic activity of a new class of compounds - Schiff's base of 3-amino-2-methyl quinazoline 4(3H) ones. *Indian J Physiol Pharmacol* 1995;**39**:169-71.

85. Ismail ST, Gopalkrishnan S, Haneeza Begum V. Biochemical modes of action of *Gmelina asiatica* in inflammation. *Indian J Pharmacol* 1997;**29**:306-9.

86. Krishnaveni M, Suja V, Vasanth S, Shyamaladevi CS. Anti-inflammatory and analgesic actions of 4', 5,6-trihydroxy-3'7-dimethoxy flavone-from *Vicoa Indianica* DC. *Indian J Pharmacol* 1997;**29**:178-81.

87. Rao KS, Mishra SH, Studies on *Circuligo orchioides Gaertn* for anti-inflammatory and hepatoprotective activities. *Indian Drugs* 1996;**33**:20-5.

88. Geetha T, Varalakshmi P, Mary Latha SR. Effect of triterpenes from *Crataeva nurvala* stem bark on lipid peroxidation in adjuvant induced arthritis in rats. *Pharmacol Res* 1998;**37**:191-5.

89. Chattopadhyay RR. Possible mechanism of anti-inflammatory action of *Azadirachta indica* A. Juss in rats, *Indian J Exp Biol* 1998;**36**:418-20.

90. Raja Naresh RA, Pillai GK, Udupa N, Chandrashekhar G. Anti-inflammatory activity of the niosome encapsulated diclofenac sodium in arthritic rats. *Indian J Pharmacol* 1994; **26**:46-8.

91. Kulkarni SK, Ninan I, Singh A. Antispasmodic activity of diclofenac and its combination with pitofenone and fenpiverinium on rat colon. *Indian J Pharmacol* 1998;**30**: 323-5.

92. Hore SK, Dumka VK, Tripathi, HC, Tandan SK, Kumar D. Modulatory role of central purinergic neurotransmitters on Brewer's yeast induced inflammation in rats. *Indian J Pharmacol* 1997;**29**:190-3.

93. Hore SK, Dumka VK, Tandan SK, Tripathi HC, Kumar D. Modulation of Brewer's yeast - induced peripheral inflammation and nociception in rats by centrally administered prostaglandins and their inhibitors. *Indian J Pharmacol* 1997; **29**:416-9.

94. Vani RR, Rawal UM. Glutathione and glutathione redox cycle in riboflavin supplemented cataractous rat lenses. *Indian J Pharmacol* 1996;**28**:93-7.

95. Sharma P, Kulshreshtha S, Sharma AL. Anti-cataract activity of Ocimum sanctum on experimental cataract. *Indian J Pharmacol* 1998;**30**:16-20.

96. Jena J, Ahmed Q, Kar PK. Effects of combined H_1 and H_2 blockers activity against histamine induced bronchospasm in guinea pigs. *Indian J Pharmacol* 1994;**26**:190-3.

97. Abraham E, Deodhar M, Natu MV, Satija VK. Bronchodilator effects of sublingual nifedipine in allergic bronchial asthma. *Indian J Pharmacol* 1994;**26**:62-3.

98. Nair AM, Saraf MN. Inhibition of antigen and compound 48/80 induced contractions of guinea pig trachea by the ethanolic extract of the leaves of *Vitex negundo* Linn. *Indian J Pharmacol* 1995;**27**:230-3.

99. Nair AM, Mugantiwar AA, Kamal KM, Saraf MN. Modulation of *in vitro* anaphylaxis by furosemide. *Indian J Pharmacol* 1997;**29**:182-6.

100. Sahani SR, Jerajani HR, Sharma K, Cooverji ND. Anti-inflammatory effects of fluticasone, a topically active synthetic corticosteroid and its combination formulations in histamine induced cutaneous responses in healthy human volunteers. *Indian J Pharmacol* 1998;**30**:245-8.

101. Nair AM, Tamhankar CP, Saraf MN. Studies on the mast cell stabilizing activity of *Vitex negunda* Linn . *Indian Drugs* 1995;**32**:277-82.

102. D'Souza RD, Bhandare PN, Fernandes N, Wahidulla S, D'Souza L. Antispasmodic action of 5-methyl benzoxazoline-2-one: A preliminary study. *Indian J Pharmacol* 1997;**29**: 327-8.

103. Gupta PP, Srimal RC, Verma N, Tandon JS. Passive cutaneous anaphylactic inhibitory and mast cell stabilizing activity of coleonol and its derivative. *Indian J Pharmacol* 1994; **26**:150-2.

104. Gupta PP, Srimal RC, Avasthi K, Garg N, Chandra T, Bhakuni DS. Anti-allergic activity of alkyl substituted pyrazolo[3, 4-d]pyrimidine (compound 88-765). *Indian J Exp Biol* 1995;**33**:38-40.

105. Venkatesan N, Punithavathi V, Chandrakasan G. Curcumin protects bleomycin induced injury in rats. *Life Sci* 1997;**61**:51-8.

106. Chatterjee A, Chaudhri S. Influence of superoxide dismutase on chromosomal observations induced by reduced glutathione. *Current Sci* 1997;**73**:693-7.

107. Kundu B, Shukla M, Khare S, Gupta PP, Singh R, Amarnath, Patnaik GK. Anti-allergic activity of some novel oligopeptides related to immunoglobulin E. *Biorganic Med Chem Lett* 1997;**9**:1191-4.

108. Singh R, Gupta PP, Singh PP. Cultivation of mouse bone marrow-derived mast cells using concanavalin A stimulated splenocyte supernatant. *Indian J Pharmacol* 1998;**30**:195-8.

109. Singh R, Nath A, Gupta PP, Shukla M, Khare SK, Kundu B. Anti-allergic/anti-asthmatic activity of oligopeptide related to IgE. *Pharmacol Res* 1998b;**37**:353-6.

110. Bhatt SR, Burande MD, Shah SM, Mehta AA, Goyal RK. Effect of chronic treatment with oestrogen and progesterone on histamine levels and histamine H_1 and H_2 receptors in rats and guinea pigs. *Pharmacol Rev Commun* 1997; **9**:113-22.

111. Kotwani A, Mehta B, Iyengar B. Diflunisal affects chick embryo neural tube development - an action not mediated by prostaglandins. *Indian J Pharmacol* 1997;**29**:117-24.

112. Biswas S, Kabir SN, Pal AK. The role of nitric oxide in the process of implantation in rats. *J Reprod Fertil* 1998;**114**: 157-61.

113. Manonmani S, Vishwanathan VP, Subramanian S, Govindasamy S. Biochemical studies on the antiulcerogenic activity of Cauvery 100, an ayurvedic formulation in experimental ulcers. *Indian J Pharmacol* 1995;**27**:101-5.

114. Jain SM, Parmar NS, Santani DD. Gastric anti-ulcer activities of calcium channel blockers in rats. *Indian J Pharmacol* 1994;**26**:29-34.

115. Jain S, Santani D. Modification of duodenal ulcer by calcium channel blockers in rats. *Indian J Pharmacol* 1996;**28**: 167-70.

116. Desai, JK, Parmar NS. Effect of sulpiride, a dopamine receptor antagonist, on experimentally induced gastric and duodenal ulcers in rats. *Agents and Actions* 1994;**42**:149-53.

117. Vanisree AJ, Mitra K, Devi S. Antiulcerogenic effect of UL-409 against experimentally induced gastric ulcer in rats. *Indian J Pharmacol* 1996;**28**:265-8.

118. Vanisree AJ, Shyamala Devi CS. Biochemical studies evaluating the antiulcerogenic potential of UL409, a herbal drug formulation on experimental peptic ulcers in rats. *Current Sci* 1997;**73**:684-91.

119. Chaudhri L, Vedasiromoni JR, Besra SE, Ganguly DK. Role of nitric oxide in gastrokinetic effects of the new benzoxepine derivative expanol hydrochloride. *Arzeneimittelforschung* 1996;**46**:923-5.

120. Rastogi L, Patnaik GK, Dikshit M. Free radicals and antioxidants status in the rat stomach after pyloric ligation induced ulcers. *Pharmacol Res* 1998;**38**:125-32.

121. Nath C, Rastogi L, Dikshit M and Patnaik GK, Saxena RC, Gupta MB. Interferon inducer Poly I: Poly C - A potent antigastric ulcer agent and inhibitor of gastric proton pump in rats. *Clin Exp Pharmacol Physiol* 1998;**25**:564-7.

122. Naidu MUR, Reddy DN, Shobha JC, Prasad VBN, Kumar TR. Endoscopic evaluation of the effects of ketorolac and diclofenac sodium on the gastroduodenal mucosa. *Indian J Pharmacol* 1996;**28**:194-5.

123. Kumar S, Kumar M, Gupta RL. Ketorolac vs. nefopam: comparison of single intravenous dose in relieving post-operative pain following laparotomy. *Indian J Pharmacol* 1996;**28**: 240-3.

124. Roy V, Gupta U, Sachdeva G, Bapna JS. Adverse drug reactions to ranitidine, famotidine and omeprazole with special reference to hepatic functions and lipid profile: A Prospective study. *Indian J Pharmacol* 1997;**29**:20-3.

125. Thakur PR, Umathe SN, The relation between centrally situated noradrenergic, adenosine-A1 and GABA-B receptors while inhibiting gastric acid secretion in pylorus ligated conscious rats. *Indian J Pharmacol* 1998;**30**:186-90.

126. Bhunia AK, Sarkar D, Das PK. Leishmania donovani attachment stimulates PKC-mediated oxidative events in bone marrow-derived macrophages. *J Eukaryot Microbiol* 1996;**43**:373-9.

127. Basu NK, Kole L, Ghosh A, Das PK. Isolation of nitric oxide synthase from the protozoan parasite, Leishmania donovani. *FEMS Microbiol Lett* 1997;**156**:43-7.

128. Sarkar K, Das PK. Protective effect of neoglycoprotein-conjugated muramyl dipeptide against donovani infection: the role of cytokines. *J Immunol* 1997;**158**:5357-65.

AUTONOMIC PHARMACOLOGY

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1. Introduction

Autonomic pharmacology or Autopharmacology has been the basis for the birth and growth of pharmacology as a separate discipline[1]. Pharmacology has undergone four revolutions during twentieth century *viz.,* chemotherapeutic revolution, biological revolution, molecular revolution and genetic revolution. Identification of neurotransmitters and characterization of different receptors have been major focus of research during biological revolution era. It was followed by discovery of second messengers and signal transduction mechanisms as a consequence of drug-receptor interaction and was called the molecular revolution. Future of pharmacology seems to be genetic revolution with the development of receptor cloning. In India research work in the field of autonomic pharmacology, though enormous during the period of review, has mostly been restricted to the characterization of receptors in different systems. Adithan[2] in his survey reported a sharp decline in the number of papers published in autonomic pharmacology (from 12.2% in 1972-75 to 5.8% in 1991-95). This may be because of limited facilities for molecular biology in the country in this area (1.1% papers in the area of molecular biology started appearing in 1991-95 as against zero in 1972-1983)[2].

This review comprises of research in autonomic pharmacology conducted by investigators in India during the period 1994-1998. Attempt has been made to make this article as comprehensive as possible and to cover the entire notable and available published references by going through resources available. Errors or omission may please be perceived as purely unintentional.

2. Characterization of autonomic receptors and the mechanisms

2.1 Dopamine receptors

2.1.1 Effect of haloperidol on aqueous humour dynamics

Medical management of glaucoma aims at reducing intraocular pressure (IOP) to normal. Out of several classes of drugs available, pilocarpine and timolol are extensively used, but adverse effects are also experienced. Dopaminergic involvement has been shown in the maintenance of IOP and dopamine antagonists have been found to reduce IOP. Haloperidol, a dopamine antagonist, appears to attract researchers for its role in glaucoma. Khosala *et al* [3,4] studied the effect of haloperidol on aqueous humor dynamics in experimental glaucoma and found it to delay the intra-ocular pressure recovery in rabbit eyes after infusion with hypertonic solution and to increase outflow facility indicating that it acts as an ocular hypotensive by reducing aqueous humor formation and also by increasing its outflow.

2.1.2 Effect of BR-16A, a herbal preparation

BR-16A with possible neuropsychiatric effects is a herbal preparation derived from Indian traditional medicine. Andrade *et al* [5] studied the effect of BR-16A on alpha-2 adrenergic, dopamine autoreceptors and dopamine postsynaptic receptor functioning. BR-16A was not found to interfere with alpha-2 adrenergic and dopamine autoreceptors functioning, but it enhanced dopamine post synaptic receptor activity.

2.1.3 Characterization of contractile response in rat anococcygeus muscle

The rat anococcygeus muscle is one of the most advantageous muscle preparations having a high degree of adrenergic, cholinergic and serotonergic innervation. Dopamine is also reported to give contractile response; but it is not clearly known whether dopamine receptor(s) are present in this muscle preparation and if so, their subtypes. Kulkarni and Sharma[6] tried to characterize dopamine-induced contractile response in rat anococcygeus muscle preparation. The experimental results suggested the presence of both DA_1 and DA_2 receptors in rat anococcygeus muscle and that dopamine also acted on adrenergic receptors to produce the contractile response of this muscle preparation.

2.1.4 Dopamine receptors in gastric and duodenal ulceration

Desai *et al*[7] studied the effect of SKF 38393, a specific dopamine D_1 receptor agonist, on pylorus ligation, and water immersion plus restraint stress induced gastric ulcers and cysteamine induced duodenal ulcers in rats. The data suggested the involvement of dopamine D_1 receptors in the anti-ulcer activity of SKF 38393, which could be largely associated with its anti-secretory effect.

Dopamine is regarded as not only an important central neurotransmitter but also an enteric neurotransmitter. Conflicting reports in the literature exist regarding the role of dopamine in the genesis of gastric and duodenal ulceration, gastric cytoprotection, gastric acid secretion, gastrointestinal motility and in the strengthening of gastric mucosal barrier. Desai *et al*[8] reviewed the role of dopamine receptor subtypes in gastric and duodenal ulceration. After taking into consideration the available reports, it appears that DA_1 and DA_2 receptors have opposing effects on gastric and duodenal ulceration, gastrointestinal motility, gastric mucosal blood flow and gastric acid secretion. While DA_2 receptor antagonists produce anti-ulcer effect, DA_1 receptor antagonists have been reported to produce ulcerogenic activity in various models of gastric and duodenal ulcers.

2.1.5. Effect of central administration of dopaminergic agents on gastric acid secretion.

Szabo[9] suggested the involvement of dopaminergic receptors in duodenal ulcer formation. To find out the role of central dopaminergic receptors in ulcer pathogenesis, Murugesan *et al*[10] assessed the effects of bromocriptine, a dopamine agonist, and haloperidol, a dopamine antagonist, on gastric acid secretion and ulcerogenesis in rats by intracerebroventricular (*icv*) route. Bromocriptine produced significant reductions in ulcerogenesis, total acidity, volume of secretion and total acid output, whereas haloperidol produced apparent increase in ulcerogenesis, total and free acidity and total acid output, suggesting the involvement of central dopaminergic receptors in gastric ulcerogenesis.

2.1.6. Pharmacological study of toad skin extract

Toad skin has attracted the attention of the scientists for its novelty. Studies carried out by Das *et al*[11] showed that toad skin extract (TSE) produced significant hyperthermia and potentiated pentobarbitone induced sleeping time in male albino mice. It produced a biphasic response, hypotension followed by hypertension in cats and the later action was blocked by atropine. TSE produced positive inotropic and negative chronotropic effect on isolated guinea pig heart. Atropine blocked TSE induced contractile response on guinea pig isolated ileum and rat fundus.

2.2 Adrenergic receptors and mechanisms

2.2.1 $Alpha_1$ adrenoceptor in benign prostate hyperplasia

Benign prostate hyperplasia (BPH) is a nonmalignant, age-related condition that affects most elderly men. Selective α_1 adrenoceptor blockade as treatment for sympathetic BPH is well documented and prazosin is one such drug. Basalingappa *et al*[12] investigated the effectiveness of prazosin in the treatment of BPH in Indian population confirming its usefulness. They found prazosin effective in the dose of 0.5 mg (morning) and 1.0 mg (night) in the treatment of patients with symptomatic BPH.

2.2.2 Disposition of norepinephrine and epinephrine in poultry

Various adrenergic mechanisms including biosynthesis, storage, release, metabolism and uptake of neurotransmitter(s) have been investigated in greater details in mammals but are poorly understood in avians in general and chicken in particular. Punia and Garg[13] studied disposition of norepinephrine and epinephrine in duodenum of white leghorn chicken concluding that the role of enzymatic degradation is almost equal to that of uptake processes for both (-)NE and (-)E and the uptake mechanisms do not appear to be an efficient route of disposition of catecholamines in duodenum.

2.3 Cholinergic receptors and mechanism

2.3.1 Cholinergic transmission in Newcastle disease virus (NDV) infection

Loknatha and Rajendra[14] investigated the role of cholinergic transmission in Newcastle disease of poultry caused by NDV widely prevalent in the many parts of the world. They found decreased acetylcholine esterase along with increased acetylcholine content during NDV infection in different brain regions suggesting that virus probably disrupts the cholinergic neurons resulting in inhibition of acetylcholine esterase and accumulation of acetylcholine content.

2.3.2 Repeated administration and delayed neurotoxicity by organophosphorus compounds

Organophosphorus compounds are widely used in agriculture and industry. These compounds have been extensively studied for neurotoxicity. Organophosphorus compound- induced delayed neurotoxicity is a syndrome which is characterized by a delay period of 4-21 days after intoxication before the clinical symptoms (ataxia and paralysis) are manifested. Hussain *et al* [15] carried out a comparative study of delayed neurotoxicity in hens following repeated administration of mipofox, sarin and parathion. Different parameters like neurotoxic esterase activity, acetylcholinesterase activity and spinal cord degeneration were studied. They concluded that repeated administration of equitoxic doses of mipofox, sarin and parathion to hens produced marked, moderate and non-delayed neurotoxicity respectively.

Ernest *et al*[16] investigated into the delayed effects of exposure to organophosphorus compounds in a group of 34 industrial workers. They found depressed pseudocholinesterase activity as well as peripheral neuropathy among the workers exposed to organophosphorus compounds.

2.3.3 Symptoms of organophosphorus poisoning and cholinesterase activity

In acute organophosphorus poisoning, both plasma cholinesterase and red blood cell cholinesterase activities are markedly decreased below the basal level. Bobba *et al*[17] conducted a study to examine the correlation, if any, between the inhibition of red blood cell cholinesterase (RBC ChE), plasma cholinesterase (PChE) and cerebrospinal fluid acetylcholinesterase (CSF Ache) and severity of symptoms in patients poisoned with organophosphorus compounds.

2.3.4 Quinolones and skeletal muscle function

The quinolones particularly the 4-quinolones are used for a wide variety of infections. Gopinathan *et al* [18] investigated the effects of quinolones *viz.* ciprofloxacin, norfloxacin, nalidixic acid and pefloxacin on skeletal muscle function using cat tibialis anterior muscle. The results suggested that quinolones probably have a weak curare like effect on neuromuscular junction similar to that of aminoglycosides.

2.3.5 Neuromuscular function in Setaria cervi and study of anthelmintics:

Setaria cervi is a cosmopolitan nematode parasite which resembles closely to human filarial worms in its response to drugs and can, therefore, be used for the screening of potential antifilarial agents. The whole worm and its nerve muscle preparation exhibit rhythmical movements when suspended in modified Ringer solution. Singh *et al*[19] studied the effects of aqueous and alcoholic extracts of the leaves of *Mallotus philippensis* on the spontaneous movement of the whole worm and nerve muscle preparation of *Setaria cervi* and the survival of microfilarae *in vitro.* Both the extracts inhibited spontaneous motility of whole worm and nerve-muscle preparation of *S. cervi* suggesting the antifilarial potential of the extracts.

2.3.6 Role of voltage sensitive calcium channels in helminths and effect of anthelmintics

Singhal *et al* [20] identified the voltage sensitive calcium channels and studied their role in regulation of spontaneous activity. Deprivation of calcium from bathing fluid resulted in gradual reduction in the amplitude and the rate of contraction till the movements ceased completely. Similar results were obtained by adding EDTA or nifedipine to the bathing solution. Acetylcholine which increases the spontaneous movements of the whole worm as well as NM preparation failed to do so either in calcium free solution or in the presence of EDTA suggesting that calcium channels can be blocked by specific blocking agents.

Ganguly *et al*[21] studied the regulation of the cholinomimetic action of acetylcholine and pyrantel pamoate by calcium channels in *S. cervi* nerve-muscle preparation The stimulant effect of pyrantel pamoate was blocked in calcium free solution and in a bath applied EDTA. Calcium channel blocker nifedipine also blocked the response of pyrantel pamoate suggesting that the absence of calcium ions or blockade of calcium channels results in the decrease in stimulatory effect shown by pyrantel pamoate in the above preparation.

Verapamil inhibits neuromuscular transmission in nerve-muscle preparation by inhibiting transmitter release or by blocking the action of acetylcholine. Lack of literature pertaining to the *in vivo* evidence led Patel *et al*[22] to study the interaction of verapamil with the neuromuscular blocker gallamine. The study used inclined screen method and rabbit head drop method and the enhancement of the action of gallamine by verapamil was suggested.

2.3.7 Alcohol and neuromuscular junction

Alcohol is an important vehicle for a number of vegetative drugs in pharmacological testing of various isolated preparations. Studies in relation to pretreatment with dantrolene, pancuronium, succinylcholine and hemicholinoum indicated that ethanol-induced contraction may be due to release of ACh or ACh like transmitter at neuromuscular junction and calcium acts as mediator to produce these effects[23]. Interactive study with pancuronium, gallamine and succinylcholine on ethanol-induced contraction in frog's rectus muscle suggested that ethanol may be acting through metactoid nicotinic receptors or by the release of acetylcholine like substance[24,25]. Kela *et al* [26] further studied interaction of gallamine with ethanol in mammalian model using rabbit head drop method and suggested that it is not necessary to adjust the dose of gallamine in alcoholic persons.

3. Interactive studies and influence of pathological conditions on autonomic receptors :

3.1 Hypertension and hyperthyroidism

3.1.1 Effect of long term treatment with atenolol

Bhugra and Gulati[27] reported that chronic treatment with atenolol produces a decrease in noradrenaline and terbutaline induced responses in isolated ventricle. However, in preparations obtained from DOCA-saline hypertensive or hyperthyroid rats chronic treatment with atenolol did not produce any change in noradrenaline induced positive inotropic responses. However, the maximal response to terbutaline was reduced. It was hypothesized that this beneficial effects of atenolol in hypertension and hyperthyroidism may be due to reduction in the number of beta receptors in the heart.

3.1.2 Effect of long term treatment with alpha-adrenoceptor blockers

Prolonged alpha adrenoceptor blockade in the ventricle is reported to produce a compensatory upregulation of beta adrenoceptor whereas chronic thyroxine treatment produces upregulation of beta adrenoceptors. Simultaneous treatment with yohimbine and thyroxine produced down regulation of beta adrenoceptors. In the aorta, chronic prazosin treatment produced changes in both control and hypertensive preparations attributable to alpha-blockade locally. In aortic strip preparations from thyroxine treated animals the changes could be secondary to those produced in the ventricle[28].

3.1.3 Effect of long term treatment with ethinyl estradiol

Chronic treatment of rat with estrogen increases sympathetic nervous tone. Bhatt and Gulati[29] reported that chronic treatment of rat with ethinyl estradiol (EE) did not alter the pD_2 values of NA in isolated aortic strip, however, it produced significant increase in pD_2 values of NA in portal vein preparation. It was concluded that chronic treatment of rat results in supersensitivity of portal vein to NA, which may be prejunctional in nature.

3.1.4 Effect of long term treatment with calcium channel blockers

Chronic treatment with calcium channel blockers (CCBs) like diltiazem, nifedipine and nimodipine in rats was found to produce an increase in the pD_2 value of NA and terbutaline (TA)[30]. However, in preparations from hypertensive or hyperthyroid rats, the maximal responses to NA and TA were decreased with reduction in pD_2 value of NA and no effect on that of TA. This phenomenon was also explained on the basis of the beneficial effects of CCBs in hypertension and hyperthyroidism might be due to down regulation of beta adrenoreceptors.

Bhugra and Gulati[31] studied the interaction of calcium channel blockers with histamine and 5-hydroxytryptamine in rat isolated aortic strip preparations. Chronic treatment with CCBs was found to completely block the contractile responses to histamine suggesting that this may be due to inhibition of the voltage dependent channels and inositol 1,4,5-triphosphate-induced release of calcium from intracellular stores, whereas, decreased contractions of the aortic strip preparation with 5-HT may be due to a decrease in 5-HT receptor density. In DOCA-hypertensive rats, responses to histamine and 5-HT were variable. In thyroxine treated rats responses to histamine were found to be abolished while that of 5-HT partially blocked.

Bhugra and Gulati[32] also found that in preparations from control and hypertensive rats chronically treated with verapamil, nifedipine and diltiazem, contractions to NA, NA and phenylephrine (PE) were partially inhibited. However, with nimodipine, there was potentiation of responses which could be related to the occurrence of various isoforms of L-type calcium channels.

There is evidence that voltage-dependent and receptor-operated Ca^{2+} channels in different tissues may vary in their sensitivity to calcium channel blockers. Bhatt and Hemavathi[33] studied the differential effects of verapamil and nifedipine on isolated vas deferense of guinea pig to various agonists and observed that verapamil and nifedipine produced concentration-dependent inhibition of the electrical field stimulation-induced contractile responses in guinea pig isolated vas deferense preparation. It was concluded that voltage-operated calcium channels are more sensitive to both the CCBs than the receptor-operated calcium channels and nifedipine to be more potent than verapamil in antagonizing receptor operated calcium channels.

3.2. Diabetes mellitus

Diabetes mellitus is the commonest endocrine disorder with a variety of complications involving kidney and nerves. Neuropathy, a related complication of diabetes mellitus, is reported to occur with an incidence of 20-40% in diabetes mellitus. It involves eyes, cardiovascular system, renal, genito-urinary tract and gastrointestinal tract.

3.2.1 Felodipine and cholinergic responses of colonic smooth muscle

Gastrointestinal tract is a prime target of diabetic autonomic neuropathy. Jaiprakash *et al*[34] studied the effect of felodipine on the cholinergic responses of the colonic smooth muscles of the streptozotocin induced diabetic rats. In both, control and diabetic rats treated with felodipine, contractile response were found to be reduced. However, in felodipine treated diabetic rats, significant response to exogenous acetylcholine was observed. They suggested further studies on the effect of felodipine on gastrointestinal motility *in vivo* in diabetic rats.

3.2.2 Felodipine, myocardial function and cholinergic responses

Cardiovascular complications of diabetes mellitus account for 80% of deaths among diabetics. Autonomic neuropathy increases the susceptibility of the diabetic myocardium to arrhythmias. Decreased contractility of diabetic myocardium is associated with intracellular calcium overload. However, the relationship between calcium levels and myocardial cholinergic responses is not known. Naga Rani *et al*[35] studied the effect of felodipine on the myocardial function and the cholinergic responses of the spontaneously working isolated heart of rats with short term streptozotocin-induced diabetes mellitus. Felodipine did not alter the blood glucose levels; but attenuated the increased cardio-osmotic ratio in the diabetics.

3.2.3 Autonomic dysfunction and peripheral vasodilatory response

Autonomic functions and peripheral vascular status have been reported to be abnormal in non-insulin dependent diabetes mellitus. Deepak *et al*[36] correlated autonomic dysfunction and peripheral vasodilator response in diabetes. They observed that patients with diabetic autonomic neuropathy do exhibit peripheral vascular dysfunction.

3.2.4 Chronic hydralazine treatment and various preparations

Hydralazine is reported to interfere with sympathetic control of cardiovascular effects. Chronic treatment with hydralazine for six weeks prevents diabetes induced cardiac dysfunction and bradycardia. Satia *et al*[37] studied the effect of chronic treatment with hydralazine in various *in vitro* preparations of rats and found that a six week treatment caused a significant increase in isoprenaline-induced positive inotropic response in rat heart, but not positive chronotropic response. The treatment also failed to alter nor-adrenaline-induced contractile effects on rat anococcygeus muscle. However, myocardial preparations from hydralazine treated rats showed an increase in adrenaline-induced relaxation. Possibly influence of hydralazine on adenylate cyclase-cyclic adenosine monophosphate (cAMP) may be responsible for such effects.

3.2.5 Sympathetic skin response and autonomic dysfunction

Jha and Nag[38] investigated the sympathetic skin response (S.S.R.) and autonomic dysfunction in diabetes. The study was conducted on 34 middle aged diabetics of either sex to compare the autonomic function in patients having and not having symptoms of dysautonomia. They showed that S.S.R. could be used as an easy, sensitive and probably an early indicator of autonomic dysfunction.

3.3 Miscellaneous

3.3.1 Beta adrenoceptor sensitization and Furosemide

Nair *et al* [39] studied the desensitization of adrenoceptors of the isolated heart trachea and its reversal by furosemide. They proposed that the reversal of desensitization might be possibly due to steroid sparing effect.

3.3.2 Interaction of pargyline with tolbutamide

Satyanarayana *et al*[40] studied the interaction of pargyline with tolbutamide in rabbits and showed that pargyline significantly increases the elimination half life and area under curve of tolbutamide resulting in prolonged hypoglycemia owing to the ability of pargyline to inhibit hepatic metabolism of pargyline.

4. Adenosine and autonomic receptors

4.1 Effect of adenosine on contractility in rat urinary bladder

Adenosine is known to produce relaxation of a variety of smooth muscles- guinea pig carotid artery, rabbit aorta, guinea pig taenia coli and urinary bladder. Keeping in view the fact that drugs producing smooth muscle relaxation inhibit the entry of Ca^{2+} into the myoplasm, Parija *et al*[41] studied the effect of adenosine on K^+ and acetylcholine stimulated contractility and 32Ca uptake in the rat urinary bladder and compared those with nifedipine. Both, adenosine and nifedipine were found to significantly inhibit the contractions elicited by K^+. The authors concluded that calcium channel blocking action of adenosine is limited to uptake through voltage operated calcium channels. Muscarinic receptor stimulation appears to be insensitive to the purine.

4.2 Adenosine and gastric acid secretion

It is well known that basal gastric acid secretory activity is largely controlled by vagus and noradrenaline is one of the centrally active neurotransmitters to inhibit gastric acid secretion. Adenosine, a putative neuromodulator/neurotransmitter has also been identified as an agent that can inhibit acid secretion. Substantial evidence indicate that GABA initiates its antisecretory effect through GABA-B receptors. Thakur and Umathe[42] studied the relation between centrally situated noradrenergic, adenosine A_1 and GABA-B receptors while inhibiting gastric acid secretion in pylorus ligated conscious rats. They showed that centrally situated adenosine A_1 receptors operate through α_1 adrenergic receptors while inhibiting gastric acid secretion and it does not bear any relation with centrally situated $GABA_B$ receptors, which also happen to inhibit gastric acid secretion.

5. Autonomic functions in cardiovascular system

5.1 Acute coronary artery occlusion

Coronary artery occlusion is known to activate cardiac autonomic receptors. It induces various cardiovascular reflex responses and autonomic nervous system plays a critical role in triggering ventricular fibrillation. Hussain and Fahim[43] studied the cardiovascular responses to phenylephrine and sodium nitroprusside during acute coronary occlusion in dogs. This study suggested that the cardiovascular reflex effects of phenylephrine and sodium nitroprusside are significantly attenuated following acute left anterior descending coronary artery occlusion and blocking any of the components of ANS changed these responsiveness.

5.2 Congestive cardiac failure

The high circulatory catecholamines and angiotensin II levels in congestive cardiac failure (CCF) is a major factor leading to death in such patients. It was thought that the use of α_2 adrenoceptor antagonists in low output cardiac failure might have rational advantage over other vasodilators. In a study conducted by Shah and Goyal[44], yohimbine, an α_2 adrenoceptor antagonist, successfully reversed the propranolol-induced decrease in L.V.P. and +dp/dtmax. Yohimbine was also found to bring back the propranolol induced decrease in carotid artery blood flow rate.

5.3 Reperfusion injury

Despite the obvious benefits of reperfusion in acute myocardial infarction, reestablishment of coronary blood flow can also elicit several biochemical, mechanical and electrophysiological consequences which are collectively called as reperfusion injury. Shekher *et al* [45] studied the role of noradrenaline uptake inhibitor desipramine in the prevention of reperfusion arrhythmias in intact rabbit heart and isolated rat heart. In intact heart studies, desipramine offered protection against reperfusion arrhythmias in dose related manner, whereas in isolated studies, 50% protection was observed in the overall incidence of arrhythmias.

Sharma and Pandhi[46] studied the effect of metoprolol and prazosin alone and in combination in reperfusion induced arrhythmias in intact rabbits. They showed that the combination of a cardioselective beta blocker metoprolol and an alpha blocker prazosin produces potentiation of effect in protecting the heart against reperfusion induced arrhythmias in intact rabbit heart.

5.4 Benzene and adrenaline induced ventricular tachycardia

Thomas *et al* [47] studied the antagonistic effects of several adrenergic blocking agents on ventricular tachycardia induced by benzene and adrenaline in rats. All the alpha and beta adrenergic blockers studied showed significant antiarrythmic effect in this model.

5.5 Cardiovascular responses to phenylephrine during acute experimental anemia

In acute experimental anemia, the cardiovascular responses to different cardioactive drugs change. Talwar *et al*[48] showed that induction of experimental anemia produced an increase in cardiac output due to an increase in heart rate and stroke volume and the effect of phenylephrine on cardiovascular responses gets significantly attenuated.

6. Newer synthetic compounds

6.1 Selective β_1-adrenoceptor antagonist with β_2 agonistic activity

A significant number of new compounds with selective and novel activity were synthesized during this period. Shah *et al*[49] reported synthesis of compound LM-2616 showing beta-1 adrenoceptor antagonistic with β_2 adrenoceptor agonistic activity. LM-2616 also produced 100% local anesthesia by filtration and surface anaesthesia method. The authors concluded that LM-2616 could be developed as a potential antiarrythmic drug.

6.2. Nonselective beta adrenoceptor antagonistic activity

Kannan *et al* [50] synthesized three compounds of phenoxypropanolamine type with chlorine substituted at para (PCP), and ortho (OCP) positions and without chlorine (NCP). All three compound reduced tachycardia induced by isoprenaline in dogs and rats. While compounds I and II significantly blocked the isoprenaline induced fall in blood pressure implying a non-selective β_1 and β_2 blocking effect in both species.

7. Traditional Indian medicine

7.1 Autonomic changes during 'OM' meditation and Yoga

Meditation and Yoga were also profoundly studied for their effects on autonomic nervous system notably by Telles *et al* [51], which is relatively new field of research . The autonomic and respiratory variables were studied in seven experienced mediators. Each subject was studied in two types of sessions- meditation (with a period of mental chanting of OM) and control (with a period of non-targeted thinking). It was found that OM meditation increased mental alertness even when physically relaxed.

Investigations on claims regarding alteration in the autonomic functions following exclusive breathing through one nostril by Telles *et al* [52] suggested that breathing selectively through either nostril could have marked activating - or relaxing effect on the sympathetic nervous system. They suggested possible alteration in metabolism due to exclusive breathing pattern for the therapeutic effects.

"The concept of yoga is helpful for treatment of bronchial asthma" has created great interest in medical research field. Khanam *et al*[53] found that after seven days yoga training nine diagnosed bronchial asthma patients indicated the reduction in sympathetic reactivity and improvement into pulmonary ventilation by way relaxation of voluntary inspiratory and expiratory muscles.

7.2 Effects of chronic terbutaline and Abana pretreatment on guinea pig isolated trachea

Bhattacharya *et al* [54] studied the effects of chronic treatment with Abana, which is a herbomineral formulation, on beta adrenoceptor mediated isolated tracheal relaxation. Effect of this formulation was studied on maximal relaxant responses to isoprenaline, terbutaline and aminophylline and they concluded that chronic Abana pretreatment in the guinea pig does not affect either beta adrenoceptor mediated tracheal relaxation or the decreased responsiveness of the tracheal smooth muscle by chronic terbutaline pretreatment.

REFERENCES

1. Rand MJ. Adrentines in autopharmacology: A biographic view with digressions into other matters. *Ann Rev Pharm Toxicol* 1993;**33**:25-44.

2. Adithan C. Pharmacological research in India. *Indian J Pharmacol* 1996;**28**:125-8.

3. Khosla P, Kothari S, Gupta MC. Effect of haloperidol, a dopamine antagonist on aqueous humour dynamics in experimental glaucoma. *Indian J Exp Biol* 1996;**34**:1152-4.

4. Khosla P, Kothari S, Gupta MC, Srivastava RK. Evaluation of haloperidol, a dopamine antagonist on intraocular pressure in experimental glaucoma. *Indian J Exp Biol* 1996;**34**:580-1.

5. Andrade C, Tony R, Udaya HB, Sureshchandra J. Effect of BR16 A on alpha-2 adrenergic, dopamine autoreceptor and dopamine post receptor functioning. *Indian J Pharmacol* 1994;**26**:292-5.

6. Kulkarni SK, Sharma A. On the characterization of dopamine-induced contractile response of rat anococcygeus muscle preparation. *Indian J Exp Biol* 1994;**32**: 176-9.

Desai JK, Goyal RK, Parmar NS. Gastric and duodenal anti-ulcer activity of SKF 38393, a dopamine D_1-receptor agonist in rats. *J Pharm Pharmacol* 1995;**47**:734-8.

8. Desai JK, Goyal RK, Parmar NS. Dopamine receptor subtypes in gastric and duodenal ulceration. *Indian J Pharmacol* 1996;**28**:129-42.

9. Szabo S. Dopamine disorder in duodenal ulceration. *Lancet* 1979;**2**:880-2.

10. Murugesan S, Kanniappan M, GowriSankar B. Effect of central administration of dopaminergic agents on gastric acid secretion and ulcerogenesis in rats. *Indian Vet J* 1998;**75**: 14-6.

11. Das M, Auddy B, Gomes A. Pharmacological study of the toad skin extract on experimental animals. *Indian J Pharmacol* 1996;**28**:72-6.

12. Basalingappa S, Ignatius XJ, Madappa KM. Clinical evaluation of prazosin in symptomatic benign prostatic hyperplasia- an Indian experience. *Indian J Pharmacol* 1997;**29**: 420-5.

13. Punia JS, Garg BD. Disposition of norepinephrine and epinephrine in duodenum of white leghorn chicken (WLH). *Indian J Pharmacol* 1994;**26**:147-9.

14. Lokanatha V, Rajendra W. Alteration in cholinergic transmission indifferent brain regions of chick during Newcastle Disease virus (NDV) infection. *Indian J Exp Biol* 1996;**34**: 479-9.

15. Husain K, Pant SC, Raza SK, Singh R, Dasgupta S. A comparative study of delayed neurotoxicity in hens following repeated administration of organophosphorus compounds. *Indian J Physiol Pharmacol* 1995;**39**:47-50.

16. Ernest K, Thomas M, Rupa V, Gnanamuthu C. Delayed effects of exposure to organophosphorus compounds. *Indian J Med Res* 1995;**101**:81-4.

17. Bobba R, Venkataraman BV, Pais P, Joseph T. Correlation between the severity of symptoms in organophosphorus poisoning and cholinesterase activity (RBC and plasma) in humans. *Indian J Physiol Pharmacol* 1996;**40**:249-53.

18. Gopinathan N, Potkar CN, Barve AS, Kshirsagar NA. Effect of quinolones on skeletal muscle function. *Indian J Pharmacol* 1995;**27**:234-40.

19. Singh R, Singhal KC, Khan NU. Antifilarial activity of *Mallotus philippensis* lam. On Setaria cervi (nematode: filarioidea) *in vitro. Indian J Physiol Pharmacol* 1997;**41**: 397-403.

20. Singhal KC, Ganguly B, Awasthi PK. Identification of voltage sensitive calcium channels in Setaria cervi (nematode: filarioidea) and their role in regulation of spontaneous activity. *Indian J Physiol Pharmacol* 1995;**39**:117-21.

21. Ganguly B, Awasthi PK, Singhal KC. Regulation of cholinomimetic action of pyrantel pamoate by calcium

channels Setaria cervi. *Indian J Physiol Pharmacol* 1996;**40**; 245-8.

22. Patel BG, Chotai NP, Shah NN. An interaction study of verapamil with the neuromuscular blocker gallamine. *Indian J Pharmacol* 1997;**29**:266-8.

23. Kela AK, Sharma AK, Mehta SC, Mehta VL, Srivastava AK. On mechanism of action of ethanol-induced contractions of frog rectus abdominis. *Indian J Exp Biol* 1994;**32**: 200-2.

24. Kela AK, Sharma AK, Mehta VL. Interactions of neuromuscular blocking agents with ethanol. *Indian J Exp Biol* 1994;**32**: 434-5.

25. Kela AK, Sharma AK, Mehta VL. Does ethanol release acetylcholine to produce contraction on isolated frog rectus abdominis? *Indian J Pharmacol* 1995;**27**:195-6.

26. Kela AK, Sharma AK, Mehta SC, Mehta VL. Interaction of muscle relaxant effect of gallamine with ethanol: *in vivo* mammalian model. *Indian J Exp Biol* 1997;**35**:302-3.

27. Bhugra P, Gulati OD. Effect of chronic atenolol treatment on responses to noradrenaline and terbutaline in isolated ventricle from hypertensive and hyperthyroid rats. *Current Science* 1998;**74**:691-5.

28. Bhugra P, Gulati OD. Interaction of alpha adrenoceptor antagonists with various agonists in isolated aorta and ventricle from normal and diseased rats. *Indian J Pharmacol* 1996;**28**:77-83.

29. Bhatt JD, Gulati OD. Effect of chronic treatment of rat with ethinyl estradiol on the sensitivity of isolated vascular preparations to noradrenaline. *Indian J Pharmacol* 1996;**28**: 113-5.

30. Bhugra P, Gulati OD. Interaction of calcium channel blockers (CCBs) with noradrenaline and terbutaline on isolated right ventricle from normal and diseased rats. *Indian J Pharmacol* 1998;**30**:82-8.

31. Bhugra P, Gulati OD. Interaction of calcium channel blockers (CCBs) with histamine and 5-hydroxytryptamine in aorta from normal and diseased rats. *Indian J Physiol Pharmacol* 1996;**40**:120-6.

32. Bhugra P, Gulati OD. Interaction of calcium channel blockers (CCBs) with different agonists in aorta from normal and diseased rats. *Indian J Physiol Pharmacol* 1996;**40**: 109-19.

33. Bhatt JD, Hemavathi KG. Differential effects of verapamil and nifedipine on isolated vas deferense of guinea pig to various agonists. *Indian J Pharmacol* 1995;**27**:94-100.

34. Jaiprakash R, Nagarani MA, Venkataraman BV. Effect of felodipine on cholinergic responses of the colonic smooth muscle of streptozotocin induced diabetic rats. *Indian J Exp Biol* 1995;**33**:297-9.

35. Nagarani MA, Venkataraman BV, Jaiprakash R. Effect of nifedipine on myocardial function and cholinergic responses in short term streptozotocin diabetes in rats. *Indian J Exp Biol* 1994;**32**:629-32.

36. Deepak KK, Godbole SA, Kochhar KP, Shah P, Kochupillai N. Autonomic dysfunction and peripheral vasodilatory response in diabetes. *Indian J Physiol Pharmacol* 1996;**40**: 325-9.

37. Satia MC, Gandhi TP, Goyal RK. Effect of chronic treatment with hydralazine on various *in vitro* preparations of rat. *Indian J Exp Biol* 1996:**34**:205-7.

38. Jha S, Nag D. Sympathetic skin response and autonomic dysfunction in diabetes. *Indian J Physiol Pharmacol* 1995; **39**:149-53.

39. Nair AM, Mungantiwar AA, Shinde UA, Saraf MN. Beta adrenoceptor desensitization of the isolated rat trachea and its reversal by furosemide. *Pharm Sci* 1997;**3**:93-7.

40. Satyanarayana S, Murlikrishna D, Krishnaiah YSR, Visweswaran D. Interaction of pargyline with tolbutamide in rabbits. *Indian J Physiol Pharmacol* 1995;**39**:71-3.

41. Parija SC, Raviprakash V, Mishra SK. Effect of adenosine on contractility and 45 Ca-uptake in rat urinary bladder. *Indian J Exp Biol* 1994;**32**:781-5.

42. Thakur PR, Umathe SN. The relation between centrally situated noradrenergic, adenosine-A1 and GABA-B receptors while inhibiting gastric acid secretion in pylorus ligated conscious rats. *Indian J Pharmacol* 1998;**30**:186-90.

43. Hussain ME, Fahim M. Cardiovascular responses to phenylepinephrine and sodium nitroprusside during acute coronary occlusion in dogs. *Indian J Physiol Pharmacol* 1994;**38**:252-8.

44. Shah GB, Goyal RK. Effect of yohimbine on congestive cardiac failure. *Indian J Pharmacol* 1994;**26**:41-3.

45. Shekher A, Sharma PL, Pandhi P, Singh M. Role of adrenaline uptake inhibition and alpha adrenoceptors in reperfusion ventricular arrhythmias *in vitro* and *in vivo*. *Indian J Exp Biol* 1996;**34**:1085-90.

46. Sharma D, Pandhi P. Effect of metoprolol and prazosin alone and in combination in reperfusion induced arrhythmias in intact rabbits. *Indian J Pharmacol* 1997;**29**:310-3.

47. Thomas GP, Naidu MUR, Tripathi RM. Varma RK. Antagonistic effects of several adrenergic blocking agents on

ventricular tacchycardia induced benzene and adrenaline in rat. *Indian J Pharmacol* 1994;**26**:52-4.

48. Talwar A, Hussain ME, Gupta CK, Fahim M. Cardiovascular responses to phenylephrine during acute experimental anaemia in anaesthetized cats. *Indian J Physiol Pharmacol* 1995;**39**:106-10.

49. Shah S, Singh N, Goyal RK, Dev A, Chhabria MT, Shishoo CJ. Pharmacological evaluation of LM-2616: a beta-1 adrenoceptor antagonist with beta-2 adrenoceptor agonist activity. *Pharm Comm* 1995;**5**:253-65.

50. Kannan J, Giridhar R, Balaraman R. Synthesis and preliminary cardiovascular activity of some phenoxypropanolamines as beta blockers. *Indian J Pharmacol* 1996;**28**: 120-2.

51. Telles A, Nagarathna R, Nagendra HR. Autonomic changes during OM meditation. *Indian J Physiol Pharmacol* 1995;**39**:418-20.

52. Telles A, Nagarathna R, Nagendra HR. Breathing through one particular nostril can alter metabolism and autonomic activities. *Indian J Physiol Pharmacol* 1994;**38**:133-7.

53. Khanam AA, Sachdev U, Guleria R, Deepak KK. Study of pulmonary and autonomic functions of asthma patients after yoga training. *Indian J Physiol Pharmacol* 1996;**40**: 318-24.

54. Bhattacharya P, Bhatt JD, Hemavathi KG, Gulati OD. Effects of chronic terbutaline and Abana pretreatment on beta adrenoceptor mediated relaxant responses of guinea pig isolated trachea. *Indian J Pharmacol* 1994;**26**:270-6.

CARDIOVASCULAR SYSTEM

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1. Introduction

Research in the area of cardiovascular system has been going on only in some centres and some of the previously active centres are no longer that active. During the period of review, there is a declining trend in this area of research. For the sake of clarity, the significant contributions made by the different laboratories in the country have been divided into the following sections.

2. Pharmacology of vascular system

2.1. Thrombosis

Thrombosis has been an area of active research during this period not only because of its clinical importance but also because of new knowledge that has accumulated the world over. A number of pharmacological and physiological approaches have been used to identify the underlying events that produce thrombosis. Free radical scavengers, platelet activating factor antagonists, cyclooxygenase inhibitors and Nitric Oxide (NO) releasing compounds have been found to be effective in preventing paralysis/death of mice and rats following thrombosis induced by intravenous administration of collagen and adrenaline[1-3]. These authors suggested the involvement of free radicals in the genesis of thrombosis. Role of neutrophils in thrombosis has also been proposed[4-6].

2.2. Hypertension

A number of studies have been published in which beneficial effects of antihypertensive drugs have been evaluated experimentally in either normal animals or in uncomplicated cases of hypertension or more commonly they were also made diabetic or some other disease was induced. Thus Satia and co-workers[7] found clonidine an effective and useful drug in rats made hypertensive with DOCA and diabetic with streptozotocin (STZ). It also reduced serum cholesterol, triglycerides and LDL-cholesterol and increased HDL-cholesterol levels along with the hypotension. Similar beneficial effects were reported with amlodipine, a calcium channel blocker, on chronic treatment of spontaneously hypertensive rats made diabetic with STZ[8]. Goyal *et al* [9] compared the relative merits of atenolol and prazosin in STZ treated rats. Streptozotocin treatment induced diabetes, hypertension, cardiomyopathy and hypercholesterolaemia after 6 weeks. Whereas atenolol reduced the blood pressure only, prazosin prevented other complications also in addition to reducing the blood pressure. Goyal and his co-workers also studied[10,11] the effect of chronic treatment of rats with lisinopril and enalapril in which hypertension was induced by DOCA and diabetes by STZ and they found that lisinopril reduced the STZ-induced weight loss, hypertension, bradycardia, hypothyroidism, hyperglycaemia and hypoinsulinaemia and also serum cholesterol, triglycerides and improved LVDP while enalapril had all these actions except that it did not have any effect on plasma glucose and insulin levels in diabetic rats. Parulekar and his colleagues[12] studied the effect of spirapril on the blood pressure of spontaneously hypertensive rats made diabetic with STZ. They found that the drug not only reduced the blood pressure but also prevented or significantly reduced other complications of diabetes like loss of weight, bradycardia, hyperglycaemia and hyper-cholestrolemia. Shah *et al*[13] recommended the use of nifedipine in the treatment of hypertension associated with diabetes mellitus since it increased insulin sensitivity and has some beneficial effects on cardiovascular parameters.

Some authors have investigated the mechanism of action of established antihypertensive drugs. Sharma *et al*[14] produced evidence that minoxidil has got agonist activity on α_2-adrenoceptors in addition to potassium channel opening property to account for its hypotensive activity. Bhugra and Gulati[15-17] studied the interaction of calcium channel blockers with different autonomic agents on rat aortic strip from normal, hypertensive and hyperthyroid animals in an effort to understand whether response to calcium channel blockers could change in the presence of disease since this group of drugs are very commonly prescribed for cardiovascular diseases. Treatment with verapamil, nifedipine and diltiazem resulted in reduced response to noradrenaline (NA), phenylephrine (PE) and dopamine (DA) in strips from normal and hypertensive rats but nimodipine potentiated these responses. However, in strips from hyperthyroid rats all the responses were reduced. The response to histamine (H) was completely blocked and to 5-HT was inhibited.These changes were explained on the basis of inhibition of the voltage-dependent calcium channels. Nimodipine may also have caused decreased density of α-adrenoceptors as an additional factor. Satia *et al*[18] studied the effect of chronic administration of hydralazine in rat *ex-vivo.* A six week treatment led to significant increase in isoprenaline-induced positive chronotropic response of heart without altering its inotropic effect. NA-induced contraction of anococcygeus muscle was not altered but adrenaline-induced relaxation of myometrial preparations was potentiated.The authors explained these results on the basis of effect of hydralazine on adenylate cyclase-cAMP system.

Gurtu and his co-workers[19] studied the effect of intravenous administration of calcium channel blockers on cardiovascular sympathetic neurotransmission. They found that only verapamil inhibited it whereas nifedipine and diltiazem did not have any effect and concluded that peripheral sympathetic inhibition contributed to its effect on the heart rate and the blood pressure.

Rathod *et al*[20] studied the effect of dietary calcium and diltiazem on the blood pressure of hypertensive rats and found that calcium supplementation in diet potentiated the effect of diltiazem and this was explained on the basis of stabilisation of ion fluxes by calcium and decrease of abnormal intracellular calcium level which was implicated in hypertension.

Puri[21] has analysed cadmium-induced hypertension in rats and suggested that the complete attenuation of the hypertensive response by verapamil could be due to ischaemic preconditioning by the drug. Lall *et al*[22] analysed the effect of cadmium on blood pressure. They administered it by *iv* as well as icv routes and found similar effects. However, the peak hypertensive effect was accompanied by raised plasma renin activity(PRA) which was blocked by captopril, losartan and captopril in combination with losartan. Central administration of Cd was not accompanied by raised PRA levels and it was abolished by centrally administered losartan but not by captopril supporting the involvement of peripheral reninangiotensin system. Talwar *et al*[23] investigated the reactivity of the cardiovascular system of cat during different stages of acute anaemia and reported that PE responses were significantly attenuated possibly due to reduced oxygen supply to myocardium and also due to release of other vasodilating factors which antagonised the effect of PE. Walia and his co-workers[24] investigated the role of genetic predisposition in offspring of normotensive and hypertensive parents and concluded that in the offspring of hypertensive parents there was a definite increase in cardiovascular reactivity on cold pressor test as measured by the rise in systolic and diastolic blood pressure. There was a possible predisposition of these subjects to hypertension in later life.

Bamosa[25], while investigating the cause of hypertension, reported that Na^+/H^+ exchanger has similar activity in portal veins of WKY and SH rats and that its blockade contracts both the preparations. It is, therefore, unlikely that increase of Na^+/H^+ exchanger activity, acting directly on vascular smooth muscle tone, could be a primary cause for hypertension.

3. Pharmacology of the heart

3.1. Ischemia reperfusion

Several investigators have studied the mechanism of injury to heart due to ischemia and the role of reperfusion in this injury since reperfusion is known to aggravate the injury. Efforts have been made to delineate the role of preconditioning of the heart in its subsequent damage due to injury since evidence for its beneficial effect exists. Seth and his colleagues[26] demonstrated the specific additional injurious component of reperfusion *per se* and the protective effect of low dose of aspirin in the prevention of at least some of the manifestations of ischemia reperfusion injury, even when it was administered late in ischemia just before reperfusion. The same group[27] demonstrated, in a canine model of ischemia-reperfusion, the cardioprotective effect of iloprost even when administered late in ischemia just before reperfusion but its combination with aspirin had no additional protective effect. Kaur *et al*[28] analysed the effect of amiloride, a Na^+/H^+ exchange inhibitor, on the cardiac preconditioning and suggested that ischaemic preconditioning and pH preconditioning produced cardioprotective effects by activating NA^+/H^+ exchange and consequent degranulation of resident cardiac mast cells. Amiloride administered during ischaemic preconditioning attenuated this effect. Parikh and Singh[29] analysed the role of endotoxin in myocardial preconditioning in rat. They proposed that cardioprotective and antiarrythmic effect of endotoxin preconditioning may be due to induction of nitric oxide synthase and subsequent increased release of NO. This may stabilise the cardiac mast cells and decrease the release of cytotoxic mediators from these cells.

Singh and his co-workers[30] provided evidence for the protective role of thromboxane (TXA_2) receptor antagonist, SQ 29548, on the ischaemic myocardium since thromboxane has a deleterious role in ischemia. TXA_2 blockade may have potential as a mode of therapy for ischaemic heart disease. Maulik *et al*[31] studied the effect of oxyfedrine administered just before reperfusion in a canine model of myocardial stunning. It was found to be effective in preventing the haemodynamic and metabolic changes associated with myocardial stunning. Kaur *et al*[32] had earlier studied the effect of nitrendipine, nimodipine and nisoldipine on isoprenaline-induced myocardial infarction in rats and observed their efficacy. Nisoldipine was found to be most effective in this respect as compared to nitrendipine and nimodipine.

Bhugra and Gulati[33] analysed the effect of several calcium channel blockers on isolated right ventricle of rats made hypertensive with DOCA or made hyperthyroid by treatment with L-thyroxine and compared with normal rat ventricle. They suggested that calcium channel blockers down-regulate β_1-receptor and may up-regulate β_2-receptors. The beneficial effects of calcium channel blockers in hypertension and hyperthyrodism may be due to down regulation of beta adrenoceptors.

Dikshit *et al*[34] demonstrated in cat heart that quinidine and curcumin pre-treatment were able to prevent the ischemia induced elevation in MDA content and LDH release. Curcumin did not prevent the increase in MPO activity while quinidine did. Tandon *et al*[35] have reported that Picroliv, a standardised active fraction isolated from roots and rhizomes of *Picrorhiza kurroa*, has significant protective activity against myocardial ischemia induced by isoproterenol or coronary ligation. The cardioprotective activity of Picroliv was comparable with other natural products (gugulip, guggulsterone, coleonol) which exerted significant activity equivalent to standard drugs such as nifedipine and propranolol. Seth and his co-workers[36] administered Lipistat comprising of equal-proportions of extracts of *Terminalia arjuna*, *Inula racemosa* Hook and latex of *Commiphora mukul* orally daily for 60 days to rats. Thereafter, the rats were subjected to isoproterenol-induced myocardial necrosis. There was significant cardioprotection in Lipistat treated animals.

Ithayarasi and Shyamala Devi[37] studied the effect of α-tocopherol on lipid peroxidation in isoproterenol-induced myocardial infarction in rats and found that it offered protection to the myocardium by preventing the inactivation of antioxidant enzymes and membrane bound enzymes thereby inhibiting peroxidation of polyunsaturated fatty acids resulting in the maintenance of myocardial membrane integrity and cellular thiol status. These effects helped the myocardium to withstand the stress of isoproterenol. Tripathi and Hegde[38] studied the effect of α-tocopherol pretreatment on the infarct size in dog following ischemia and reperfusion and found significant beneficial effect on infarct size and lipid peroxidation. Tripathi *et al*[39] further reported that reperfusion in previously ischaemic myocardium caused injury de *novo*. Combination of superoxide dismutase and acidified sodium nitrite reduced the infarct size and lipid peroxidation after ischemia and reperfusion. They suggested different mechanisms by which superoxide dismutase and acidified sodium nitrite, a liberator of nitric oxide, may offer cardioprotection probably by a direct action either on endothelium or on myocardial cells or both.

3.2. Arrhythmia

Tripathi and Varma[40] showed that all the α- and β-adrenergic blocking agents examined had significant antiarrythmic effect in the benzene and adrenaline model in rat. The relative potencies of α-blockers were: prazosin > yohimbine > phenoxy-benzamine > phentolamine while that of β-blockers were: labetalol > propranolol > practolol > sotalol. Gautam *et al*[41] determined the potency of selected drugs in abolishing ventricular arrhythmias induced by coronary artery ligation. It was in the following descending order: verapamil, sotalol, propranolol and quinidine. Shekhar *et al*[42] investigated the role of sympathetic system in the ventricular arrhythmia induced by coronary artery ligation followed by reperfusion in intact rabbit heart and isolated rat heart. They used desipramine as the catecholamine uptake inhibitor and found that it consistently reduced reperfusion induced ventricular arrhythmias in both *in vivo* and *in vitro* models possibly by inhibiting catecholamine efflux. On the other hand, dose-response curve of phenylephrine (selective α-1 agonist) shifted towards right during both ischaemia and reperfusion. Bhattacharya *et al*[43] studied the effect of calcium channel blocking drugs on focal ventricular arrhythmias induced in cat by subepicardial adrenaline infusion. The aim of the study was to understand the effect of drugs at the site of arrhythmogenesis. Verapamil was found to be more effective than nifedipine in antagonising adrenaline induced ventricular arrhythmias.

3.3. Role nitric oxide (NO) in the cardiovascular system

Studies[44,45] on the involvement of NO/Arginine/cGMP pathway in the central cardiovascular regulation in cat suggested that this pathway was involved in the regulation of cardiovascular system. Heart rate (HR) was found to be increased following *icv* administration of SNP and L-arginine. In addition, L-arginine increased the blood pressure, while SNP was found to decrease it. Further studies in hypertensive rats indicated interrelationship between BP and brain NOS activity. Hypertensive rats were prepared by coarctation of the abdominal aorta in between the right and left kidneys. NOS activity was significantly reduced in the brain (medulla and hypothalamus) of hypertensive rats. Following treatment with anti-hypertensive drugs captopril, nifedipine and centhaquin, (a centrally acting antihypertensive CDRI compound), normalisation of brain NOS activity was observed.

Verma *et al*[46] demonstrated significant hypotension and bradycardia by local application of sodium nitroprusside on the ventral surface of medulla and its blockade by methylene blue. It was suggestive of the possible involvement of NO-cGMP mechanism in central cardiovascular regulation.

Reactive oxygen species (ROS: superoxide radicals, hydrogen peroxide and hydroxyl radicals) considerably influence endothelium of intact aortic ring contractions from normotensive animals. NO has been found to attenuate the effect of ROS during noradrenaline induced contractions in the endothelium intact rings. However, in the endothelium denuded rings, no such effect was observed. It is evident that in the endothelium, NO interacts with ROS during aortic ring contraction. In the renal hypertensive rats, attenuation in the acetylcholine induced NO release and augmentation in the ROS generation seems to play an important role in the modulation of vasoreactivity[47,48].

4. New drugs

4.1. Natural products

A number of natural products (either formulation or extract) have been studied during the period of this review. Whereas Shaila *et al*[49] reported the anti-atherogenic activity of *Terminalia arjuna* in experimentally induced atherosclerosis in rabbits, Bhatia *et al*[50] reported the effect of the aqueous extract of bark of *Terminalia arjuna* on coronary flow in isolated perfused rabbit heart preparation. They found an increase in the coronary flow which supported its clinically reported antianginal activity and its use in Ayurveda as cardioprotective agent. Fahim *et al*[51] found Ajmaloon to be a potent antihypertensive drug which produced dose-dependent fall in blood pressure of rabbit and monkey and did not interfere with the normal baroreceptor mediated reflex regulatory mechanism and should thus be free from the problem of postural hypotension.

Bopanna *et al*[52] studied the cell culture extract of *Hemidesmus indicus* in normal and the hyperlipidemic rats. They reported that administration of atherogenic diet concurrently with *Hemidesmus indicus* lowered the levels of cholesterol and lipids in liver, heart and serum while faecal excretion of cholesterol and phopholipids were significantly increased.

S-allyl cysteine sulphoxide is a major sulphur containing amino acid in plants of Lilliaceae and Cruciferae families. Garlic and onion are rich sources. Bopanna *et al* [53] reported a significant antagonism of deleterious metabolic effects of monosodium glutamate in rats fed with atherogenic diet. The same group[54] also demonstrated that S-allyl cysteine sulphoxides produced a significant antioxidant effect in rats getting atherogenic diet and monosodium glutamate. Chopra and Singh[55] found that rutin could limit the infarct size in rats and concluded that this capacity of rutin could be due to its ability to impair the generation of reactive oxygen species.

Bopanna *et al* [56] reported significant antidiabetic and antihyperlipaemic activity in neem seed kernel (NS) powder (500 mg/kg/day, *p.o.*) alone or in combination with glibenclamide (0.50 mg/kg/day, *p.o.*) in alloxan diabetic rabbits. The activity of the combination was much greater than NS alone. However, liver hexokinase activity was increased by all the treatments. Koley and Lal[57] studied the effect of *Azadiracta indica* (neem) leaf extract on the ECG and blood pressure of rat and suggested that there was no involvement of muscarinic and histaminergic receptors in neem leaf extract induced fall in blood pressure.

Gupta *et al* [58] reported that psyllium husk has an effective lipid lowering activity in non insulin dependent diabetes mellitus with hyperlipidaemia patients. It is a well tolerated adjunct to diet.

Bhatt *et al* [59] studied the effect of Abana, a proprietary preparation, on normal and ethinyl estradiol-induced hypertensive rats and found that it protected rats against the effects of the ethinyl estradiol probably by its sympathetic blocking property. In normal rats also it lowered blood pressure after 3 week treatment.

4.2. Synthetic drugs

There are very few reports concerning the synthesis of new potential drugs for cardiovascular activity. Thus Kannan *et al* [60] described the synthesis and preliminary cardiovascular activity of some phenoxypropanolamines as β-adrenergic antagonists. These compounds with chlorine substituted at para and ortho positions produced blockade of isoproterenol-induced tachycardia in rats which was similar to the effect produced by propranolol. Reddy[61] reported some new thrombolytic drugs for the treatment of acute myocardial infraction such as prourokinase, saruplus, alteplase, k1 k2 Pu and staphylokinase which have shown promise in animal models of arterial and venous thrombosis and also in pilot scale clinical studies in patients with myocardial infarction. However, more clinical trials are needed to determine whether these novel recombinant thrombolytic agents show improved efficacy and fibrin specificity with minimal bleeding tendencies

5. Miscellaneous

Gaur and his colleagues[62] studied the platelet functions and lipid profile within 24 hours of an attack of Transient Ischeuic Ahoces (TIA), thrombotic and haemorrhagic stroke patients and concluded that platelet hypofunction has a role in the pathogenesis of hypertensive putaminal haemorrhage while in patients of transient ischaemic attack and thrombotic stroke, lipids may be major contributing factor in cerebral atherogenesis. Sreelatha Kumari *et al* [63] reported elevated level of serum glycosyminoglycans (GAG) associated with hypomagnesia in patients of proven coronary artery disease and thrombotic stroke. Serum lipid level was normal in majority of the patients indicating that elevated serum GAG may be an even more reliable indicator of atherosclerosis than serum total cholesterol or total LDL cholesterol. Autopsy samples of carotid artery and aorta which had atheroma showed significantly higher GAG compared to samples which showed no atheroma. Serum Mg levels were significantly lower in coronary artery disease and thrombotic stroke patients as compared to controls. Mg deficiency may be one of the factors involved in the increased level of GAG.

Kumari *et al* [64] while investigating role of platelets and neutrophils in thrombosis, found a factor in the neutrophil supernatant which inhibited platelet aggregation that may have a role in preventing intravascular thrombosis.

Hussain and Fahim[65] suggested that both parasympathetic (vagal) and sympathetic efferents are involved in the hypertensive and hypotensive effects of phenylephrine and sodium nitroprusside during acute coronary artery occlusion in dog. The vagal component seems to play relatively larger role.

Shah and Goyal [66] found that yohimbine has a beneficial effect in congestive cardiac failure associated with the blockade of post synaptic α_2-adrenoreceptors in vascular smooth muscles and some positive inotropic actions of yohimbine causing an increase in the cardiac output.

Julka *et al* [67] investigated the mechanism of adriamycin-induced cardiotoxicity and provided evidence that it could evoke release of free radicals which eventually caused the peroxidation of membrane lipids and a concomitant decrease in various antioxidants that led to membrane dysfunction.

6. Concluding remarks

Review of research in the area of cardiovascular pharmacology in five years from 1994-98 makes it amply clear that the emphasis has shifted from classical pharmacology towards the work on *in vitro* system and at molecular level. The latter trend is still not fully reflected during the period of review but is gradually increasing. Some of the publications have come out in the best International journals and that speaks of the quality of research that is going on in the country. However, it is disturbing to note that the number of active research centres in this field has gone down and so is the number of active research workers. It is to be hoped that international quality research will increase further and many young researchers will take interest in cardiovascular pharmacology which is so vital for medical progress.

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REFERENCES

1. Seth P, Kumari R, Dikshit M, Srimal RC. Effect of PAF-antagonists in the models of thrombosis. *Thrombosis Res* 1994;**76**:503-12.
2. Seth P, Kumari R, Dikshit M. Alteration in the free radical generation and nitric oxide release from rat polymorphonuclear leukocytes following thrombosis. *Thrombosis Res* 1997;**87**:279-88.
3. Kumari R, Seth P, Dikshit M, Srimal RC. Alterations in the bioanti-oxidants following thrombosis. *Free Radical Biol Med* 1994;**17**:481-4.
4. Kumari R, Seth P, Dikshit M. Involvement of bioanti-oxidants in thrombosis in mice. *Redox Report* 1995;**7**:191-5.
5. Dikshit M, Kumari R. Modulation of platelet activation by mediators released from neutrophils. *Hematology* 1997;**2**: 39-53.
6. Dikshit M, Chari S, Seth P, Kumari R. Interaction of nitric oxide synthase inhibitors and their D-enantiomers with rat polymorphonuclear leukocyte luminol dependent chemiluminescence response. *Brit J Pharmacol* 1996;**119**:578-82.
7. Satia MC, Damani RR, Goyal RK. Beneficial effects of clonidine in streptozotocin-induced diabetes and DOCA-hypertensive rats. *J Pharm Pharmacol* 1997;**49**:1030-5.
8. Srinivasan PS, Hakim ZS, Santani DD, Goyal RK. Effects of chronic treatment with amlodipine in streptozotocin-diabetic and spontaneously hypertensive rats. *Pharmacol Res* 1997;**35**:423-8.
9. Goyal RK, Bangaru RA, Lakkad NB, Rao MV. Effect of chronic treatment with atenolol and prazosin in streptozotocin induced diabetic rats. *Indian J Physiol Pharmacol* 1996;**40**:220-4.
10. Sevak AR, Goyal RK. Effect of chronic treatment with lisinopril on cardiovascular complications in streptozotocin diabetic and DOCA hypertensive rats. *Pharmacol Res* 1996;**34**:201-9.
11. Goyal RK, Satia MC, Bangaru RA, Gandhi TP. Effect of long term treatment with enalapril in streptozotocin diabetic and DOCA hypertensive rats. *J Cardiovasc Pharmacol* 1998;**32**: 317-22.
12. Parulekar AA, Hakin ZS, Santani DD, Goyal RK. Effect of chronic treatment with spirapril on biochemical parameters in streptozotocin-diabetic and spontaneously hypertensive rats. *Indian J Exp Biol* 1997;**35**:1182-6.
13 Shah TS, Satia MC, Gandhi TP, Bangaru RA, Goyal RK. Effects of chronic nifedipine treatment on streptozotocin-induced diabetic rats. *J Cardiovasc Pharmacol* 1995;**26**: 6-12.
14. Sharma N, Mehta AA, Santani DD, Goyal RK. Evidence for alpha 2-adrenoceptor agonist activity of minoxidil. *J Pharm Pharmacol* 1997;**49**:935-7.
15. Bhugra P, Gulati OD. Interactions of alpha-adrenoceptor antagonists with various agonists in isolated aorta and ventricle from normal and diseased rats. *Indian J*

Pharmacol 1996;**28**:77-83.

16. Bhugra P, Gulati OD. Interaction of calcium channel blockers with different agonists in aorta from normal and diseased rats. *Indian J Physiol Pharmacol* 1996;**40**:109-19.

17. Bhugra P, Gulati OD. Interaction of calcium channel blockers (CCBs) with histamine and 5-hydroxytryptamine in aorta from normal and diseased rats. *Indian J Physiol Pharmacol* 1996;**40**:120-6.

18. Satia MC, Gandhi TP, Goyal RK. Effect of chronic treatment with hydralazine on various *in vitro* preparations of rat. *Indian J Exp Biol* 1996;**34**:205-7.

19. Gurtu S, Mukherjee D, Shukla S. Evidence for a role of inhibition of sympathetic neurotransmission in the cardiovascular effects of intravenously administered verapamil. *Pharmacol Res* 1995;**31**:383-6.

20. Rathod SP, Shah N, Balaraman R. Antihypertensive effect of dietary calcium and diltiazem, a calcium channel blocker on experimentally induced hypertensive rats. *Indian J Pharmacol* 1997;**29**:99-104.

21. Puri VN. Effect of verapamil on cadmium induced hypertension in rats. *Indian J Exp Biol* 1996;**34**:1268 -70.

22. Lall SB, Peshin SS, Gulati K, Khattar S, Das N, Seth SD. Involvement of renin-angiotensin system in hypertensive effect of cadmium in rats. *Indian J Exp Biol* 1997;**35**:338-41.

23. Talwar A, Hussain ME, Gupta CK, Fahim M. Cardiovascular responses to phenylephrine during acute experimental anaemia in anaesthetised cats. *Indian J Physiol Pharmacol* 1995;**39**:106-10.

24. Walia V, Ahuja L, Kaur P. Cardiovascular reactivity to cold pressor test in offspring of normotensive and hypertensive parents. *Indian J Med Res* 1994;**99**:38-41.

25. Bamosa, AO. Blockade of Na^+/H^+ exchanger contracts the portal vein of spontaneously hypertensive and Wistar Kyoto rats. *Indian J Physiol Pharmacol* 1997;**41**:154-8.

26. Seth SD, Maulik M, Manchanda SC, Maulik SK. Role of aspirin in modulating myocardial ischemia reperfusion injury. *Agent Action* 1994;**41**:151-5.

27. Seth SD, Manchanda SC, Maulik SK. Lack of any additional benefit in combining aspirin with iloprost in a canine model of myocardial reperfusion injury. *Prostaglandins* 1997;**53**:291-303.

28. Kaur H, Parikh B, Sharma A, Singh M. Effect of amiloride a Na^+/H^+ exchange inhibitor on cardioprotective effect of ischaemic preconditioning: possible involvement of resident cardiac mast cells. *Pharmacol Res* 1997;**36**:95-102.

29. Parikh V, Singh M. Role of nitric oxide in the protective effect of endotoxin induced myocardial preconditioning. *Indian J Pharmacol* 1998;**30**:112-3.

30. Singh U, Seth SD, Manchanda SC, Seth S. Protective actions of thromboxane receptor antagonist, SQ 29548, on the ischaemic myocardium: morphological and hemodynamics effects. *Prostaglandins Leukotrienes Essent Fatty acids* 1997;**56**:105-10.

31. Maulik SK, Seth SD, Maulik M, Manchanda SC. Oxyfedrine in myocardial stunning. *Indian J Exp Biol* 1997;**35**:1214-17.

32. Kaur AH, Singh J, Srivastava RK, Mathur SK. Effect of nitrendipine, nimodipine, nisoldipine on experimentally induced myocardial infarction in rats. *Indian J Exp Biol* 1995;**33**:420-3.

33. Bhugra P, Gulati OD. Interaction of calcium channel blockers (CCBs) with noradrenaline and terbutaline on isolated right ventricle from normal and diseased rats. *Indian J Pharmacol* 1998;**30**:82-8.

34. Dikshit M, Rastogi L, Shukla R, Srimal RC. Prevention of ischemia induced biochemical changes by curcumin and quinidine in the cat heart. *Indian J Med Res* 1995;**101**:31-5.

35. Tandon S, Rastogi R, Shukla R, Kapoor NK, Srimal RC, Dhawan BN. Protective activity of Picroliv from Picrorhiza kurroa against myocardial ischemia induced by coronary ligation or isoprotenol. *Med Sci Res* 1995;**23**:515-8.

36. Seth SD, Maulik M, Katiyar CK, Maulik SK. Role of Lipistat in protection against isoproterenol induced myocardial necrosis in rats: a biochemical and histopathological study. *Indian J Physiol Pharmacol* 1998;**42**:101-6.

37. Ithayarasi AP, Shyamala Devi CS. Effect of α-tocopherol on lipid peroxidation in isoproterenol- induced myocardial infarction in rats. *Indian J Physiol* 1997;**41**:369-76.

38. Tripathi Y, Hegde BM. Effect of α-tocopherol pre-treatment on infarct size following 90 minutes of ischemia and 4 hours of reperfusion in dogs. *Indian J Physiol Pharmacol* 1997;**41**: 241-7.

39. Tripathl Y, Hegde BM, Raghubir CV. Effect of superoxide dismutase and acidified sodium nitrite on infarct size following ischemia and reperfusion in dogs. *Indian J Physiol Pharmacol* 1997;**41**:248-56.

40. Tripathi RM, Varma RK. Antagonistic effects of several adrenergic blocking agents on ventricular tachycardia induced by benzene and adrenaline in rat. *Indian J Pharmacol* 1994;**26**:52-4.

41. Gautam CS, Sharma PL, Pandhi P. Differential effects of selected antiarrythmic drugs on coronary artery ligation - induced ventricular arrhythmias on the right or left sides. *Methods Find Exp Clin Pharmacol* 1995;**17**:327-31.

42. Shekhar A, Sharma PL, Pandhi P. Singh M. Role of noradrenaline uptake inhibition and alpha-adrenoreceptors in reperfusion induced ventricular arrhythmias *in vivo* and *in vitro*. *Indian J Exp Biol* 1996;**34**:1085-90.

43. Bhattacharyya T.K, Mukhopadhyaya M, Debnath PK. Effect of calcium-channel blocking drugs on focal ventricular arrhythmias induced by subepicardial epinephrine infusion in cats. *Indian J Exp Biol* 1998;**36**:311-14.

44. Hegde LG, Shukla R, Dikshit M, Srimal RC. A study on the central cardiovascular regulation in the cat by L-Arginine-EDRF/NOcGMP pathway. *Arch Int Pharmacodyn Ther* 1994;**328**:155-64.

45. Hegde LG, Shukla R, Srimal RC, Dikshit M.: Alteration in the brain nitric oxide synthase activity in coarctation model of hypertension in rats. *Pharmacol Res* 1997;**36**:109-14.

46. Verma S, Raghubir R, Patnaik GK. Nitric oxide modulation of cardiovascular responses from ventral surface of medulla in cat. *Indian J Pharmacol* 1997;**29**:125-8.

47. Srivastava P, Hegde LG, Patnaik GK, Dikshit M. Role of endothelial derived reactive oxygen species and nitric oxide in norepinephrine-induced rat aortic ring contractions. *Pharmacol Res* 1998;**38**:265-74.

48. Hegde LG, Srivastava P, Kumari R, Dikshit M. Alterations in the vasoreactivity of hypertensive rat aortic rings: Role of nitric oxide and superoxide radicals. *Clin Exp Hypertension* 1998;**20**:885-902.

49. Shaila HP, Udupa SL, Udupa AL. Hypolipidemic activity of three indigenous drugs in experimentally induced atherosclerosis. *Int J Cardiology* 1998;**67**:119-24.

50. Bhatia J, Bhattacharya SK, Mahajan P, Dwivedi S. Effect of Terminalia arjuna on coronary flow - An experimental study. *Indian J Pharmacol* 1998;**30**:118.

51. Fahim M, Khan MSY, Hamid HA. Effect of ajmaloon on the baroreceptor-heart rate reflexes in anaesthetised rabbits and monkeys. *Indian J Physiol Pharmacol* 1995;**39**: 101-5.

52. Bopanna KN, Bhagyalakshmi N, Rathod SP, Balaraman R, Kannan J. Cell culture derived Hemidesmus indicus in the prevention of hypocholesterolemia in normal and hyperlipidemic rats. *Indian J Pharmacol* 1997;**29**:105-9.

53. Bopanna KN, Balaraman R, Nadig RS. Antioxidant status of S-allyl cysteine suphoxide on monosodium glutamate potentiated atherogenesis. *Indian J Pharmacol* 1998;**30**:73-81.

54. Bopanna KN, Balaraman R, Nadig RS. Role of S-allyl cysteine suphoxide in antagonising deleterious metabolic effects of monosodium glutamate in rats on antherogenic diet. *Indian J Pharmacol* 1998;**30**:152-62.

55. Chopra K, Singh M. Involvement of oxygen free radicals in cardioprotective effect of Rutin: a naturally occurring flavonoid. *Indian J Pharmacol* 1994;**26**:13-18.

56. Bopanna KN, Kannan J, Gadgil S, Balaraman R, Rathod SP. Antidiabetic and antihyperlipaemic effects of NEEM seed kernel powder on alloxan diabetic rabbits. *Indian J Pharmacol* 1997;**29**:162-7.

57. Koley K, Lal J. Pharmacological effects of Azadiracta indica (Neem) leaf extract on the ECG and blood pressure of rat. *Indian J Physiol Pharmacol* 1994;**38**:223-5.

58. Gupta RR, Agrawal CG, Singh GP, Ghatak A. Lipid lowering efficacy of psyllium hydrophilic mucilloid in non insulin dependent diabetes mellitus with hyperlipidaemia. *Indian J Med Res* 1994;**100**:237-41.

59. Bhatt JD, Panchakshari UD, Hemavathi KG, Gulati OD. Effect of Abana, an ayurvedic preparation on ethinyl estradiol-induced hypertension in rats. *Indian J Pharmacol* 1998;**30**:399-403.

60. Kannan J, Giridhar R, Balaraman R. Synthesis and preliminary cardiovascular activity of some phenoxypropanolamines as beta blockers. *Indian J Pharmacol* 1996;**28**:120-22.

61. Reddy DS. Newer thrombolytic drugs for acute myocardial infraction. *Indian J Exp Biol* 1998;**36**:1-15.

62. Gaur SPS, Garg RK, Agarwal S, Kar AM, Srimal RC. Platelet functions and lipid profile within 24 hours following an attack of TIA, thrombotic and haemorrhagic stroke. *Indian J Med Res* 1994;**99**:259-63.

63. Sreelatha Kumari KT, Augustine J, Leelamma S, Kurup PA, Ravikumar A *et al.* Elevated serum glycosyminoglycans with hypomagnesemia in patients with coronary artery disease and thrombotic stroke. *Indian J Med Res* 1995;**101**: 115-9.

64. Kumari R, Singh MP, Seth P, Dikshit M. Inhibition of platelet aggregation by a factor present in the rat peripheral neutrophil supernatant. *Thrombosis Res* 1998;**91**:75-82.

65. Hussain ME, Fahim M. Cardiovascular responses to phenylephrine and sodium nitroprusside during acute coronary occlusion in dogs. *Indian J Physiol Pharmacol* 1994; **38**:252-8.

66. Shah GB, Goyal RK. Effect of Yohimbine in congestive cardiac failure. *Indian J Pharmacol* 1994;**26**:41-3.

67. Julka D, Saini S, Gill KD. A possible mechanism for adriamycin induced cardiotoxicity. *Indian J Exp Biol* 1994; **32**:146.

STATUS REPORT ON CHEMOTHERAPEUTIC AGENTS

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1. Introduction

Infectious diseases account for a major part of the morbidity profile of our country. An enormous amount of money is spent on antimicrobial agents used for treatment and prophylaxis. Hundreds of thousands of individuals are afflicted with tuberculosis, leprosy, malaria, filariasis *etc.* Therefore, research in infectious diseases and treatment modalities of the same should become a national priority.

This report documents the status of research done in India under the broad area of chemotherapy during the years 1994 to 1998. Most of the work cited in this report has been done in departments of Pharmacology, from medical, veterinary, colleges of Pharmacy and research institutions. In this context the term chemotherapy has been taken in the broader sense to include all categories of work related to drugs and used in the treatment bacterial, viral, protozoal, fungal and parasitic diseases including cancer in humans and livestock. Therefore we have also included the synthesis, preliminary screening and assay methods of these drugs.

This report has been categorized into three major sections. Basic and applied research form the first two sections while the third is a compilation of review articles on this topic.

2. Basic research

2.1. Chemical synthesis

Ten new 1,2-disubstituted - 4 - [(pyridine - 3 yl)methylene]-imidazolin - 5 - (4H)ones were synthesized and screened for their antibacterial and antifungal activities by Rama Sarma *et al* [1]. The same group characterized a few more compounds in the same class and found some of them to have antibacterial and antifungal activity[2,3]. The synthesis and subsequent antimicrobial evaluation of 5-oxoimidazololaminopyrazole-4 carboxaldehydes and their Schiff's bases showed significant antibacterial and antifungal activity of some compounds [4]. Twenty five new pyrimidine compounds have been synthesised out of which eight have potential anticancer activity and one has moderate anti-HIV properties[5].

2.2. Screening tests/assay methods

The *in vitro* antimicrobial activity of seventeen heterocyclic compounds were tested against *E.coli, B.subtilis* and *C.albicans* and the MICs determined by Reddy *et al* [6]. Estimation of drugs in biological fluids is costly and needs sophisticated equipment. Prema Gurumurthy and co-workers have evolved simple spectrofluorimetric and microbiological assay methods for the estimation of ofloxacin in biological fluids[7].

2.3. Antimicrobial properties of plant products

The cytotoxic and anti-tumor properties of ethanolic extract of *Bacopa monnieri* (L.) Penn or Brahmi, as it is commonly called, has been described by Kumar and collegues[8] using *in-vitro* and *in vivo* models. There was dose-dependent inhibition of transformed cell lines and the altered haematological parameters were restored to a near normal state by this extract. The same team also described the anti-tumor effect of *Berberis asiatica* on Dalton's lymphoma ascites tumor cells and solid tumor in Swiss mice[9] and proved that the ethanolic extract of the root enhanced mean survival time and restored altered haematological parameters to normal in the mice. The *in vivo* tumor inhibitory effect of *Plumbago rosea* was studied on experimental mouse tumours. The radiosensitizing effects of the alcoholic extract of root was found to enhance the effects of radiation on the tumour[10] even though the extract possessed only a weak anti tumour effect.

The possible mechanism of anthelmintic action of palasonin, a lactone compound from *Butea frondosa* seeds on *Ascaridia galli* was suggested[11]. Palasonin inhibited the glucose uptake and depleted the glycogen content in the presence of glucose, indicating that palasonin affects the energy generating mechanism of the parasite. Antidermatophytic activity of aqueous and ethanolic extracts *Azadiratchta indica* leaves was tested *in vitro* [12]. The ethanolic extract was found to be more active than the aqueous one inhibiting 90% of the isolates (MIC90) at a concentration of 100 μg/ml.

The protective effect of Picroliv, prepared from the alcoholic extract of the root and rhizome of *Picrorhiza kurroa* in *Leishmania donovani* infections was studied in golden hamster[13]. A marked hepatoprotective effect of Picroliv in terms of biochemical markers and a significant antileishmanial activity implying that it can be utilized as an adjunct to chemotherapy or in combination therapy of kala azar along with Sodium stibogluconate, thus enhancing the efficacy of antileishmanials.The antifungal activity of certain Indian plant extracts[14] was studied along with the screening of many plants for antimicrobial properties[15-17].

2.4. Studies on sensitivity and resistance patterns

Multiple antibiotic resistance index and its reversal in *Pseudomonas aeruginosa* was studied[18]. The incidence of postoperative wound infection and their antibiogram was described for a teaching hospital[19].

2.5. Formulations/effects of chemotherapeutic agents on animal models

Ravikumar *et al*, studied the distribution of etoposide - loaded hydrophilic albumin microspheres in mice and concluded that they have a distinct advantage over the intravenous injection of the pure drug[20].The response of a transplantable mouse tumour to AK-2123 was studied. Rao and Umadevi worked out the feasibility of using the drug alone or along with hydrallazine, local hyperthermia and local irradiation[21] on a transplantable mouse tumor, Sarcoma 180. The results suggest that this multimodality treatment offer the possibility of further reduction in the doses of individual agents and in the adverse effects on normal tissues without compromising on its anti-tumor cure effect.

Altered tissue distribution and decreased neuromuscular toxicity of the common anticancer drug vincristine was seen when the drug molecules were encapsulated within niosomes[22]. The reduction of the toxic effects of bleomycin was also altered by niosomal encapsulation. It was found that though pulmonary and intestinal toxicity was less, there were no significant effects on the peripheral smear and bone marrow cells[23]. The onchostatic effect of melatonin and combined melatonin-tamoxifen therapy of mammary tumors in mice has been experimentally studied, concluding that melatonin and low dose tamoxifen had no additive or synergistic effect on the prevention of second generation of mammary tumors and that melatonin needs to be evaluated for an adjuvant role in chemotherapy[24]. The influence of red chilli on lipids and bile acids in different tissues in experimental colon cancer was studied[25], concluding that levels of cholesterol increased while phospholipids and free fatty acid levels decreased.

Subacute toxicity studies of Fenvalerate, a highly potent pyrethroid used to control ectoparasites in domestic animals was tested in broiler chicks. It was concluded that the fowl is comparatively resistant to fenvalerate toxicity[26].

The dosage and frequency of administration of liposomal amphotericin prepared using soya phosphatidylcholine and cholesterol was determined using Aspergillus murine model[27]. Liposomal amphotericin B was more effective than free amphotericin B on an equal dose basis when given therapeutically. A large single dose of the liposomal formulation was more effective both prophylactically and therapeutically than two divided doses of the same. It was concluded that a large single dose was more effective than two divided doses. The toxicological profile of aureofungin - a plant antifungal antibiotic was studied in rats[28]. This drug, which is used in agriculture for controlling plant fungal infection causes only mild toxic symptoms at higher doses which are reversible following its withdrawal.

3. Applied research

3.1. Studies related to specific diseases

3.1.1. Tuberculosis

The longitudinal trends in serum levels of mycobacterial secretory and cytoplasmic antigens during chemotherapy of patients with pulmonary tuberculosis was studied[77].

Liv-100, a herbal preparation, was tested for its hepatoprotective effects against isoniazid, rifampicin and pyrazinamide induced hepatotoxicity in rats. Saraswathy *et al* showed that simultaneous administration of Liv-100 protects against hepatotoxicity by reducing lipid peroxidation and restoring antioxidant defense system[78].

The susceptibility of *Mycobacterium marinum* to amoxicillin and clavulanic acid was studied both in-vitro and in-vivo concluding that amoxicillin and clavulanic acid alone were effective and combination with beta lactams did not offer any added advantage in the case of *M.marinum*[79]. Paul & De tested the comparative *in vitro* activity of fluoroquinolones in sixty clinical isolates of *M.tuberculosis.* Out of the four drugs which were tested, ofloxacin and ciprofloxacin were found to be most active[80]. Streptomycin sulphate, and rifampicin in combination with levamisole hydrochloride was highly effective in the treatment of rabbits infected with *Mycobacterium paratuberculosis*[81].

The prevalence of proven tuberculosis in renal transplant and dialysis patients is 5% in each. Hence a double blind placebo controlled trial of primary isoniazid prophylaxis in these patients was conducted which showed a trend towards protection from TB but suffered from a high drop-out rate on account of high prevalence of viral hepatitis[82]. A controlled clinical trial of oral short course regimens in the treatment of sputum positive pulmonary tuberculosis has been carried out in 1203 patients[83]. It was concluded that a fully unsupervised ethambutol containing regimen given daily for eight months was found to be very effective even in the presence of isoniazid-resistant bacilli, whereas though the twice weekly regimens had a 100% response rate the relapse rate was also high. A randomised clinical trial of short course chemotherapy in abdominal tuberculosis concluded that the six months course was as effective as a standard 12 months course[84]. The effect of additional oral ofloxacin in the treatment of multidrug resistant tuberculosis was tested in 19 patients[85]. Drug resistance to the first line of drugs was also reported[86].

The kinetics of isoniazid and acetylisoniazid in serum and urine of patients with pulmonary primary complex being treated with the intermittent regimen have been studied[87].

The acetylator status and its role in the hepatotoxicity induced by anti-tubercular drug treatment was studied and it was proved that the acetylator phenotype probably has no contributory role in this adverse effect[88]. A case control study was undertaken to identify risk factors for hepatotoxicity from antitubercular drugs[89].

3.1.2. Malaria

Picroliv, a standardized preparation of iridoid glycosides from *Picrorhiza kurrooa* provided significant protection against the depletion of reduced glutathione levels in liver and brain of *Mastomys natalensis* infected with *Plasmodium berghei* [90]. α-terthiophene and erythrosin-B are naturally occurring plant secondary metabolites which have phototoxic properties against Anopheles and Culex larvae. Such studies using biodegradable and eco-friendly compounds in mosquito control is of great importance in India[91]. The antimalarial activity of *Artemisia japonica, Artemisia maritima* and *Artemisia nilegarcia* was tested as a part of efforts to identitfy new antimalarial agents[92]. All three compounds prolonged survival time of mice in the Rane test and inhibited schizont maturation in a concentration dependent manner.

The *in-vitro* chloroquine resistance of *Plasmodium falciparum* in Vellore, Tamil Nadu has been documented by Venkatesan *et al*[93] because malaria itself has not been endemic for many years in Vellore. Drug resistance is a major problem with diseases like malaria and patterns change from time to time in various parts of the country. Poly inosinic-polycytidylic acid stabilized with polylysine and carboxymethyl cellulose (Poly ICLC) enhanced the antimalarial activity of chloroquine when tested against multidrug resistant *Plasmodium yoelii* nigeriensis infected mice[94]. The results suggested a possible involvement of cytochrome P-450 mediated mechanism in the enhancement of the antimalarial activity.

The effect of arteether α/β on uncomplicated falciparum malaria cases in an endemic tea garden area in upper Assam proved that 150mg *i.m.* once a day of the drug for three consecutive days was extremely effective as a schizonticidal drug[95]. The drug also exhibited gametocytocidal action. The efficacy of chloroquine versus amodiaquine was studied in 338 subjects from the states of Assam and Meghalaya. Amodiaquine had no advantage in the treatment of these patients with *P.falciparum* infections in chloroquine resistant areas[96]. The relapse pattern in five villages in Gujarat was studied for *P.vivax* [97]. The efficacy of sulfadoxine - pyrimethamine in chloroquine resistant falciparum malaria in Bombay was established[98].

3.1.3. Leprosy

The incidence rate of sensory and motor impairments during and after multidrug therapy (MDT) are reported for a prospective cohort of 1621 patients who had no nerve damage at registration. Sensory and motor loss increased with age and both were high among multibacillary patients as compared with paucibacillary patients[99]. The addition of immunotherapy in the form of a vaccine to multibacillary leprosy patients was found to benefit patients on multidrug therapy[100]. Untreated borderline lepromatous/ lepromatous leprosy patients benefited by a combination of immunotherapy and chemotherapy[101]. Ofloxacin containing treatment regimens were evaluated in lepromatous leprosy[102]. The addition of ofloxacin to the standard WHO recommended multidrug - therapy regimen for multibacillary patients may reduce the present duration of therapy. Ofloxacin may also be considered as an alternative drug in rifampicin - resistant cases or where rifampicin is contraindicated. Clofazimine may have a protective role against neuritis / Type 1 reaction and nerve damage in patients with leprosy[103]. The clinical and histopathological activity in paucibacillary leprosy patients after fixed duration multidrug therapy was studied[104]. The treatment of leprous nerve abscesses with rifampicin and isoniazid indicated the need for medical management to supplement surgical intervention in these cases[105]. Low dose Convit vaccine was tried as an adjunct to multidrug therapy and a six year follow-up study was reported from Calcutta[106,107]. Though all patients belonging to the test and the control groups showed clinical cure and bacteriological negativity within two years, immunologic potentiation as assessed by the lepromin test and leucocyte migration inhibition test was better in the test group which received low dose Convit vaccine plus multiple drug therapy. The presence of viable *M.leprae* in skin and peripheral nerves after two years of multidrug therapy in patients with multibacillary leprosy was reported from South India [108]. The efficacy and complications of fixed duration therapy in leprosy was studied in 360 patients with multibacillary leprosy[109]. The effectiveness of multidrug therapy in multibacillary leprosy was assessed[110]. The absence of relapse within 4 years was noted in those patients treated for two years with multidrug therapy[111].

Leucocytopenia after rifampicin and ofloxacin therapy in leprosy was observed[112] suggesting that patients receiving ofloxacin should be monitored for constitutional symptoms suggestive of this complication, since it is reversible with cessation of the drug. The incidence of deformity in leprosy patients treated with multidrug therapy has been collated with a view to plan services for the prevention and management of deformity[113].

Relapses during long term follow-up with drug susceptible *M.leprae* among multibacillary leprosy patients treated with multidrug therapy regimens are reported[114]. Follow-up of multi drug treatment of leprosy patients revealed that the incidence of leprosy among household contacts is still high when compared to that of the total population[115]. Ulnar abscesses were found four months after paucibacillary patients were released from control with multidrug therapy[116].

3.1.4. Filariasis

The efficacy and safety of the combination of a single dose of ivermectin and diethylcarbamazine(DEC) was evaluated in Brugian filariasis[29]. The combination of ivermectin and DEC demonstrated a microfilaricidal effect superior to that of either drug used alone, both in the initial rapid clearance of microfilariae and in sustaining the effect for one year.

3.1.5. Kala-azar

A randomized open sequential trial to select the dose of aminosidine and sodium stibogluconate for the treatment of Kala-azar was carried out[30]. The combination of aminosidine 12mg/Kg/d and sodium stibogluconate 20mg/Kg/d for 20 days appears to be an effective and safe replacement of sodium stibogluconate alone for more than 40 days. Atovaquone alone or with fluconazole as oral therapy was tested in a clinical trial for Indian kala-azar[31]. Allopurinol as an additive to quinine brings about faster eradication and clinical remission than with quinine alone in this disease[32]. The response of patients with Indian visceral Leishmaniasis treated with interferon-gamma and pentavalent antimony was also evaluated[33].

3.1.6. Whipworm

Garg *et al* [34] determined the efficacy of ivermectin and closantel against natural bubaline whipworm infection in buffalo calves. Their results showed that both drugs are equally effective for the condition.

3.1.7. Cancer

Vinblastine and danazol therapy in steroid resistant childhood chronic idiopathic thrombocytopenic purpura has been evaluated[35]. Perioperative chemotherapy in patients with oral cancer was evaluated in a prospective randomized trial[36]. Etoposide with cisplatin was considered superior to vinblastine with cisplatin because of greater efficacy, decreased toxicity and better compliance in children with malignant germ cell tumors[37]. Neoadjuvant chemotherapy prior to radiotherapy in locally advanced cancer of the cervix[38] and in locally advanced breast cancer[39] results in a high response rate and no increase in the toxicity to subsequent radiotherapy. However, there was no significant difference in overall and disease-free survival. Tamoxifen therapy benefits postmenopausal women with breast cancer with a likely decrease in risk of cardiovascular risk factors[40]. The feasibility of non-cisplatin based induction chemotherapy in advanced head and neck cancer was evaluated[41].

3.1.8. Miscellaneous

High *H.pylori* eradication rates were acheived using amoxycillin or clarithromycin based treatment regimens in combination with lansoprazole and secnidazole [42]. Fewer side-effects, better compliance and low cost favoured the amoxycillin - based therapy. The efficacy of antimicrobial drug therapy in non-dysenteric persistent diarrhoea in a community setting was determined[43]. The initial experience with cefaperazone monotherapy for serious infections in hospitalized patients has been described[44].

3.2. Drug interactions

Many Ayurvedic formulations contain Trikatu (Piperine) which inhibits drug metabolizing enzymes. Therefore, the pharmacokinetic profile of isoniazid in rabbits and its modification by Trikatu was studied. The bioavailability of isoniazid was reduced by Trikatu[45]. The bioavailability of diclofenac sodium was reduced by rifampicin pretreatment for a period of six days[46].

3.3. Studies in livestock

Aminoglycoside antibiotics are still the mainstay of gram negative aerobic infections in humans and animals. A study of the pharmacokinetics of gentamicin following single-dose parenteral administration to goats was done[47,48]. Amikacin kinetics in goats was studied by Uppal *et al*[49] who worked out the dosage which would not produce oto- or nephropathic effects. A study on the pharmacokinetics of the long acting sulphonamide sulfamethoxypyridazine in goats by Garg & Uppal[50] revealed that the subcutaneous route may be preferred to the intramuscular route. These authors have also worked out the ideal dosage regimen in goats which can be given easily under field conditions and yet maintain adequate serum concentrations when given twice a day[51].

Studying the pharmacokinetics of a single dose of cephalexin in male calves after intravenous and subcutaneous administration, Garg *et al*[52] confirmed the priming and maintenance doses by the different routes. The pharmacokinetics of pefloxacin[53], parbendazole[54] and triclabendazole[55] was studied in goats and/or sheep. The disposition kinetics of oxytetracycline in plasma, milk and uterine fluid after intravenous administration in female buffaloes was studied[56].

3.4. Drug utilization and pharmacoepidemiological studies

The auditing of prescriptions to study utilization of antimicrobials in a tertiary care hospital underlined the need for routine audits to improve rational use of antibiotics[57]. Drug utilization of antimicrobial agents in a 800 bedded hospital was done documenting the fact that 34%of antimicrobial use was empirical, 27% directed and 32% for prophylactic use[58]. An audit of the practice of antibacterial prophylaxis in general surgery wards was undertaken over a four week period underscoring the fact that antibiotics are inappropriately prescribed in this setting[59].

3.5. Adverse drug reactions

Ciprofloxacin induced bradycardia was reported as a single case report from Srinagar[61]. The appearance of new cysticercosis nodules with albendazole therapy has been documented in a 16 year old girl [62]. The familial tendency in hypersensitivity reactions to co-trimoxazole has been documented[63].

3.6. Case reports/series

A short course of ciprofloxacin at a higher dose for one week was found to be effective in the treatment of enteric fever in 18 patients[64]. The stool cultures remained free of salmonella on follow-up. The decrease in duration of treatment with ciprofloxacin has important therapeutic implications since the emergence of resistance to other drugs.

The treatment of entomophthoromycosis caused by the fungus *Conidiobolus coronatus* with ketoconazole and a saturated solution of potassium iodide has been reported[65].

The efficacy of a single oral dose of closantel against paramphistomosis in sheep has been documented by Bhushan *et al*[66].

4. Review articles

The fluoroquinolones are now being widely used all over India for all types of infections leading to development of resistance. In this direction the 2-pyridones are emerging as a very potent and novel class of agents which may probably take the place of the fluoroquinolones. This has been reviewed by Barar[67]. The rationale behind the use of cotrimoxazole has been written up as a commentary by Thomas[68]. A review of the work done on *Withania somnifera* or Ashwagandha as it is commonly known on its radiosensitizing effects and use in cancer chemotherapy gives a comprehensive account of studies done on this aspect[69]. Though the mechanism of action of this compound is not known, studies so far indicate that *W. somnifera* could prove to be a good natural source of a potent and relatively safe radiosensitizer/chemotherapeutic agent. The role of integrins in cancer biology was reviewed by Chatterjee[70]. The therapeutic values of onion and garlic have been reviewed in which the antibiotic effects of these plants have been described[71]. The current status of artemisnin in malaria has been elaborated in detail in a review by Velecha & Tripathi[72]. Fluoroquinolones have been hailed as the antimicrobial agents of the 90's[73]. The present day management of pulmonary tuberculosis[74], the treatment of clinical rabies in man[75] and the role of adjuvant therapy in colorectal cancer[76] have been the focus of a review articles by various authors.

5. Conclusion

From the review it is evident than many of the scientists do not consistently work in the same area of chemotherapy. Hence, in-depth study of the problems faced in India is sadly lacking. Long-term planning of projects is essential to achieve knowledge which can be quickly translated into clinically useful information.

REFERENCES

1. Rama Sarma RGVS, Suresh B, Reddy MV. Synthesis and antimicrobial activity of 1,2-Disubstituted-4-[(Pyridine-3-YL) Methylene] Imidazolin-5(4H)-Ones. *Indian Drugs* 1997;**34**:17-20.
2. Rama Sarma GVS, Reddy MV. Synthesis and Antimicrobial activities of 1,2-Disubstituted-4-[(Benzimidazole-2-yl)Methylene] Imidazolin-5(4H)-Ones. *Indian J Pharm Sci* 1997;**59**:236-9.
3. Rama Sarma GVS, Reddy MV. Synthesis and Biological activities of 1,2-Disubstituted-4-[(Benzoxazole-2-yl)Methylene] Imidazolin-5(4H)-Ones. *Indian J Hetero Chem* 1997;**7**:21-24.
4. De B, Sarma RGVS. Synthesis and antimicrobial evaluation of 5-oxoimidazolylaminopyrazole-4-carboxaldehydes and their Schiff's bases. *Indian J Pharm Sci* 1998; **60**:136-
5. Debi M. Synthesis of some new pyrimidine derivatives as potential anticancer and anti-HIV agent. *Indian J Exp Biol* 1997;**35**:1208-13.
6. Reddy YSR, Sama Venkatesh, Misra US, Suresh B, Azam AMd, Sethuraman M. An *in vitro* study on the antimicrobial effect of select heterocyclic nitrogen compounds. *Anc Sci Life* 1995;**15**:137-9.
7. Gurumurthy P, Ramachandran G, Kumar HAK, Venkatesan P, Chandrasekaran V, Narayanan PR. Simple spectrofluorimetric and microbiological assay methods for the estimation of ofloxacin in biological fluids. *Indian J Pharmacol* 1998;**30**:263-6.
8. Kumar EP, Elshurafa AA, Elango K, Subburaju T, Suresh B.

Cytotoxic and anti-tumour properties of Ethanolic Extract of Bacopa Monnieri(L.) Penn. *Anc Sci Life* 1998; **17**:228-34.

9. Kumar EP, Elshurafa AA, Elango K, Subburaju T, Suresh B. Anti-tumour effect of Berberis Asiartica Roxb. Ex. Dc. on Dalton's Lymphoma Ascite. *Anc Sci Life* 1998;**17**:290-9.

10. Uma Devi P, Solomon EF, Sharada AC. *In vivo* tumor inhibitory and radiosensitizing effects of an Indian medicinal plant, Plumbago rosea on experimental mouse tumors. *Indian J Exp Biol* 1994;**32**:523-8.

11. Kumar D, Mishra SK, Tandan SK, Tripathi HC. Possible mechanism of anthelmintic action of palasonin on ascaridia galli. *Indian J Pharmacol* 1995;**27**:161-6.

12. Pankajalakshmi V. Venugopal, Taralakshmi V. Venugopal. Antidermatophytic activity of neem (*Azadirachta indica*) leaves *in vitro*. *Indian J Pharmacol* 1994;**26**:141-3.

13. Mittal N, Gupta N, Saksena S, Goyal N, Roy U, Rastogi AK. Protective effect of Picroliv from *Picrorhiza kurroa* against *Leishmania donovani* infections in Mesocricetus auratus. *Life Sci* 1998;**63**:1823-34.

14. Dutt BK, Rahman I, Das TK. Antifungal activity of Indian plant extracts. *Mycoses* 1998;**41**:535-6.

15. Ahmed I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. *J Ethnopharmacol* 1998;**62**:183-93.

16. Perumal Samy R, Ignacimuthu S, Sen A. Screening of 34 Indian medicinal plants for antibacterial properties. *J Ethnopharmacol* 1998;**62**:173-82.

17. Valsaraj R, Pushpangadan P, Smitt UW, Adsersen A, Nyman U. Antimicrobial screening of selected medicinal plants from India. *J Ethnopharmacol* 1997;**58**:75-83.

18. Paul s, Bezbaruah RL, Roy MK, Ghosh AC. Multiple antibiotic resistance (MAR) index and its reversion in Pseudomonas aeruginosa. *Lett Appl Microbiol* 1997;**24**:169-71.

19. Murthy R, Sengupta S, Maya N, Shivananda PG. Incidence of post operative wound infection and their antibiogram in a teaching and referral hospital. *Indian J Med Sci* 1998; **52**:553-5.

20. Ravikumar HR, Dhanaraj SA, Rajendran D, Rajeev Dube, Suresh B. Distribution of etoposide-loaded hydrophilic albumin microspheres in mice. *Drug Dev Indian Pharm* 1996;**22**:1005-8.

21. Rao SBS, Umadevi P. Multimodality treatment using AK-2123, hydralazine, radiation and hyperthermia on a transplantable mouse tumour. *Indian J Med Res* 1996;**104**:182-9.

22. Parthasarathi G, Udupa N. Altered biological distribution and decreased neuromuscular toxicity of niosome encapsulated vincristine. *Indian J Exp Biol* 1996;**34**:124-30.

23. Naresh RRA, Udupa N, Uma Devi P. Toxicity and antitumor activity of niosomal bleomycin in tumor bearing mice. *Indian J Exp Biol* 1996;**34**:764-72.

24. Kothari A, Borges A, Kothari L. Chemoprevention by melatonin and combined melatonin-tamoxifen therapy of second generation nitroso-methylurea-induced mammary tumours in rats. *Eur J Cancer Preven* 1995;**4**:497-500.

25. Chitra S, Sabitha K, Nalini N, Menon VP. Influence of red chilli on lipids and bile acids in different tissues in experimental colon cancer. *Indian J Exp Biol* 1994;**32**:793-6.

26. Majumder S, Chakraborty AK, Mandal TK. Subacute toxicity of fenvalerate in broiler chicks: Concentration, cytotoxicity, and biochemical profiles. *Indian J Exp Biol* 1994;**32**:752-6.

27. Kotwani RN, Gokhale PC, Kshirsagar NA, Pandya SK. Optimizing dosage regimens of liposomal amphotericin B using *Aspergillus murine* model. *Indian J Pharmacol* 1996; **28**:88-92.

28. Dhuley JN, Shanbhag V, Raman PH, Mujumdar AM, Naik SR. Toxicological profile of aureofungin:A potent plant antifungal antibiotic. *Indian J Exp Biol* 1995;**33**:857-60.

29. Shenoy RK, George LM, John A, Suma TK, Kumaraswami V. Treatment of microfilaraemia in asymptomatic brugian filariasis: the efficacy and safety of the combination of single doses of ivermectin and diethylcarbamazine. *Ann Trop Med Parasitol* 1998;**92**:579-85.

30. Thakur CP, Bhowmick S, Dolfi L, Olliaro P. Aminosidine plus sodium stibogluconate for the treatment of Indian kala-azar: a randomized dose-finding clinical trial. *Transac Royal Soc Trop Med Hygiene* 1995;**89**:219-23.

31. Sundar S, Kumar P, Makharia M, Goyal A, Rogers M, Gibbs D, Murray H. Atovaquone alone or with fluconazole as oral therapy for Indian kala-azar. *Clin Infect Dis* 1998;**27**: 215-6.

32. Sarma PS, Mandal AK, Khamis HJ. Allopurinol as an additive to quinine in the treatment of acute complicated falciparm malaria. *Am J Trop Med Hygiene* 1998;**54**:454-7.

33. Sundar S, Singh VP, Sharma S, Makharia MK, Murray HW. Response to interferon-gamma plus pentavalent antimony in Indian visceral leishmaniasis. *J Infect Dis* 1997;**176**: 1117-9.

34. Garg SK, Chauhan PPS, Bhushan C, Katoch R. Efficacy of ivermectin and closantel against whipworm infection in buffalo calves. *Indian Vet J* 1998;**75**:82-3.

35. Choudhry VP, Kashyap R, Ahlawat S, Pati HP. Vinblastine and danazol therapy in steroid resistant childhood chronic idiopathic thrombocytopenic purpura. *Int J Heamtol* 1995;**61**:157-62.

36. Rao Rs, Parikh DM, Parikh HK, Bhansali MB, Deshmane VH, Fakih AR. Perioperative chemotherapy in patients with oral cancer. *Am J Sur* 1994;**168**:262-7.

37. Nair R, Pai SK, Saikia TK, Nair CN, Kurkure PA, Gopal R, Sampat MS, Advani SH. Malignant germ cell tumors in childhood. *J Sur Oncol* 1994;**56**:186-90.

38. Kumar L, Grover R, Pokharel YH, Chander S, Kumar S *et al.* Neoadjuvant chemotherapy in locally advanced cervical cancer: two randomised studies. *Aust NJ Med* 1998;**28**:387-90.

39. Singh G, Singh DP, Gupta D, Muralikrishna BV. Neoadjuvant chemotherapy in locally advanced breast cancer. *J Sur Oncol* 1996;**61**:38-41.

40. Thangaraju M, Kumar K, Gandhirajan R, Sachidanandam P. Effect of tamoxifen on plasma lipids and lipoproteins in postmenopausal women with breast cancer. *Cancer* 1994;**73**:659-63.

41. Kumar S, Datta NR, Ahuja RC, Mali HR, Agarwal GN, Ayyagari S. Feasibility of non-cisplatin-based induction chemotherapy and concurrent chemoradiotherapy in advanced head and neck cancer. *Acta Oncol* 1996;**35**:721-5.

42. Ahuja V, Dhar A, Bal C, Sharma MP. Lansoprazole and secnidazole with clarithromycin, amoxycillin or pefloxacin in the eradication of Helicobacter pylori in a developing country. *Alimen Pharmacol Therap* 1998;**12**:551-5.

43. Bahl R, Bhandari N, Bhan MK, Saxena M, Bagati A. Efficacy of antimicrobial treatment in non-dysenteric persistent diarrhoea in a community setting. *Acta Paedia* 1996;**85**:1290-4.

44. Shah SS, Deshpande AK, Kaundinya DV. Cefaperazone monotherapy for serious infections in hospitalized patients -an initial Indian experience. *J Assoc Physicians India* 1995;**43**:398-9.

45. Karan RS, Bhargava VK, Garg SK. Effect of trikatu (piperine) on the pharmacokinetic profile of isoniazid in rabbits. *Indian J Pharmacol* 1998;**30**:254-6.

46. Surya Kumar J, Mamidi RNVS, Chakrapani T, Krishna DR. Rifampicin pretreatment reduces bioavailability of diclofenac sodium. *Indian J Pharmacol* 1995;**27**:183-5.

47. Garg SK, Verma SP, Uppal RP. Pharmacokinetics of gentamicin following single-dose parenteral administration to goats. *Br Vet J* 1995;**151**:453-7.

48. Uppal RP, Verma SP, Garg SK. Pharmacokinetics and renal excretion of gentamicin in goats after intravenous administration. *Indian J Pharmacol* 1998;**30**:47-50.

49. Uppal RP, Verma SP, Verma V, Garg SK. Comparative pharmacokinetics of amikacin following a single intramuscular or subcutaneous administration in goats (Capra hircus). *Vet Res* 1997;**28**:565-70.

50. Garg SK, Uppal RP. Bioavailability of sulfamethoxypyridazine following intramuscular or subcutaneous administration in goats. *Vet Res* 1997;**28**:101-4.

51. Garg SK, Uppal RP, Riviere JE. Serum disappearance and urinary excretion of sulfamethoxypyridazine in goats. *Revue Elev Med Vet Pays Trop* 1994;**47**:215-8.

52. Garg SK, Chaudhary RK, Srivastava AK. Pharmacokinetics of cephalexin in calves after intravenous and subcutaneous administration. *Acta Veterinaria Hungarica* 1996;**44**:195-201.

53. Roy BK. Pharmacokinetics of pefloxacin in goats. *Indian J Pharmacol* 1997;**29**:435-6.

54. Lahon LC, Gupta PK. Pharmacokinetics of parbendazole in goats. *Indian J Pharmacol* 1994;**26**:235-6.

55. Sanyal PK. Pharmacokinetic study of triclabendazole in sheep and goat using a high performance liquid chromatography method. *Indian J Pharmacol* 1994;**26**:200-3.

56. Jayachandran C, Singh MK, Banerjee NC. Disposition kinetics of oxytetracycline in plasma, milk and uterine fluid after *i.v.* administration in female buffaloes. *Indian J Pharmacol* 1995;**27**:30-3.

57. Gupta N, Sharma D, Garg SK, Bhargava VK. Auditing of prescriptions to study utilization of antimicrobials in a tertiary hospital. *Indian J Pharmacol* 1997;**29**:411-5.

58. Srishyla MV, Nagarani MA, Venkataraman BV. Drug utilization of antimicrobials in the in-patient setting of a tertiary hospital. *Indian J Pharmacol* 1994;**26**:282-7.

59. Srishyla MV, Rani MA, Damodar S, Venkataraman BV, Jairam N. A preliminary audit of practice-antibacterial prophylaxis in general surgery in an Indian hospital setting. *Indian J Physiol Pharmacol* 1994;**38**:207-10.

60. Barar KV, Barar RV. Sulphonamide-like hypersensitivity reactions from some apparently safe common drugs with identical moieties. *Indian J Pharmacol* 1998;**30**:273-4.

61. Dhobi GN, Shafiqa TA. Ciprofloxacin induced bradycardia: A case report. *Indian J Pharmacol* 1994;**26**:164.

62. Anand KS, Abraham J, Gowda S, Ratnakar C. Appearance of new cysticercosis nodules with albendazole theapy.

Indian J Pharmacol 1994;**26**:165.

63. Mohanasundaram J, Rajaram S, Mohanasundaram S. Familial tendency in hypersensitivity reactions to cotrimoxazole. *J Indian Med Assoc* 1998;**96**:21-2.

64. Mathai D, Kudva GC, Keystone JS, Kozarsky PE, Jesudason MV *el al.* Short course ciprofloxacin therapy for enteric fever. *J Assoc Physicians India* 1993;**41**:428-30.

65. Mukhopadhyay D, Ghosh LM, Thammayya A, Sanyal M. Entomophthoromycosis caused by conidiobolus coronatus: clinicomycological study of a case. *Auris Nasus Larynx* 1995;**22**:139-42.

66. Bhushan C, Garg SK, Johri DK. Efficacy of closantel against natural paramphistomosis in sheep - A case report. *Indian Vet J* 1996;**73**:1262-3.

67. Barar KV. 2-Pyridones emerging as a most potent novel class of antimicrobial agents: possible future development in relation to fluoroquinolone-resistance. *Indian J Pharmacol* 1998;**30**:63-7.

68. Thomas M. Cotrimoxazole Rationale - commentary. *Drugs Ther Perspect* 1997;9:12-4.

69. Uma Devi P. *Withania somnifera* Dunal(Ashwagandha): Potential plant source of a promising drug for cancer chemotherapy and radiosensitization. *Indian J Exp Biol* 1996;**34**:927-32.

70. Chatterjee A. Integrins-new frontier in cancer biology. *Indian J Exp Biol* 1996;**34**:923-6.

71. Augusti KT. Therapeutic values of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.). *Indian J Exp Biol* 1996;**34**:634-40.

72. Valecha N, Tripathi KD. Artemisinin: current status in malaria. *Indian J Pharmacol* 1997;**29**:71-5.

73. Sharma AK, Khosla R, Kela AK, Mehta VL. Fluoroquinolones: Antimicrobial agents of the 90's. *Indian J Pharmacol* 1994;**26**:249-61.

74. Kundu AK, Kundu AK. Present-day management of pulmonary tuberculosis. *J Indian Med Assoc* 1995;**93**:144-8.

75. Dutta JK, Dutta TK, Ganj BB. Treatment of clinical rabies in man: drug therapy and other measures. *Int J Clin Pharmacol Ther* 1994;**32**:594-7.

76. Parshad R. Adjuvant therapy in colorectal cancer. *Trop Gastro* 1997;**18**:139-44.

77. Sethna KB, Mistry NF, Dholakia Y, Antia NH, Harboe M. Longitudinal trends in serum levels of mycobacterial secretory (30 kD) and cytoplasmic (65 kD) antigens during chemotherapy of pulmonary tuberculosis patients. *Scand J Infec Dis* 1998;**30**:363-9.

78. Saraswathy SD, Suja V, Gurumurthy P, Shyamaladevi CS. Effect of Liv.100 against antitubercular drugs(isoniazid, rifampicin and pyrazinamide) induced hepatotoxicity in rats. *Indian J Pharmacol* 1998;**30**:233-8.

79. Chakrabarti A, Bhattacharya CP, Acharya DP, Chakrabarty AN, Ghosh K, Dastidar SG. *In vitro* & *in vivo* experimental susceptibility of mycobacterium marinum to augmentin. *Indian J Med Res* 1996;**104**:281-3.

80. Karak (Paul), De PK. Comparative *in vitro* activity of fluoroquinolones against mycobacterium tuberculosis. *Indian J Med Res* 1995;**101**:147-9.

81. Mondal D, Sinhal RP, Gupta MK. Effect of combination therapy in Mycobacterium paratuberculosis infected rabbits. *Indian J Exp Biol* 1994;**32**:318-23.

82. John GT, Thomas PP, Thomas M, Jeyaseelan L, Jacob CK, Shastry JCM. A Double-blind randomized controlled trial of primary isoniazid prophylaxis in dialysis and transplant patients. *Transplantation* 1994;**57(11)**:1683-4.

83. Anonymous. A controlled clinical trial of oral short-course regimens in the treatment of sputum-positive pulmonary tuberculosis. Tuberculosis Research Centre. *Inter J Tubercul Lung Dis* 1997;**1(6)**:509-17.

84. Balasubramanian R, Nagarajan M, Balambal R, Tripathy SP, Sundararaman R *et al.* Randomised controlled clinical trial of short course chemotherapy in abdominal tuberculosis: a five-year report. *Inter J Tubercul Lung Dis* 1997;**1**:44-51.

85. Sharma SK, Guleria R, Jain D, Chawla TC, Saha P *et al.* Effect of additional oral ofloxacin administration in the treatment of multidrug-resistant tuberculosis. *Indian J Chest Dis All Sci* 1996;**38**:73-9.

86. Varaiya A, Gogate A. Drug resistance to the first line of antitubercular regimen (a preliminary report). *Indian J Publ H* 1998;**42**:126-30.

87. Seth V, Seth SD, Beotra A, Semwal OP, D'monty V, Mukhopadhya S. Isoniazid and acetylisoniazid kinetics in serum and urine in pulmonary primary complex with intermittent regimen. *Indian Paediatr* 1994;**31**:279-85.

88. Singh J, Garg PK, Thakur VS, Tandon RK. Antitubercular treatment induced hepatotoxicity: does acetylator status matter? *Indian J Physiol Pharmacol* 1995;**39**:43-6.

89. Pande JN, Singh SP, Khilnani GC, Khilnani S, Tandon RK. Risk factors for hepatotoxicity from antituberculosis drugs: a case-control study. *Thorax* 1996;**51**:132-6.

90. Ramesh Chander, Kapoor NK, Dhawan BN. Picroliv affects ç-glutamyl cycle in liver and brain of *Mastomys natalensis* infected with *Plasmodium berghei*. *Indian J Exp*

Biol 1994;**32**:324-7.

91. Sharma A, Goel HC. Some naturally occurring phytophototoxins for mosquito control. *Indian J Exp Biol* 1994;**32**:745-51.

92. Valecha N, Biswas S, Badoni V, Bhandari KS, Sati OP. Antimalarial activity of *Artemisia japonica, Artemisia maritima* and *Artemisia nilegarica. Indian J Pharmacol* 1994;**26**:144-6.

93. Venkatesan EA, Mathai E, Sudarsanam A, Sitaram U, Thomas M *et al.* In-vitro chloroquine resistance of P.falciparum in Vellore, Tamil Nadu. *Indian J Malariol* 1994;**31**:39-41.

94. Awasthi A, Mehrotra S, Bhakuni V, Dutta GP, Levy HB, Maheshwari RK. Poly ICLC enhances the antimalarial activity of chloroquine against multidrug-resistant *Plasmodium yoelii* nigeriensis in mice. *J Inter Cyto Res* 1997;**17**:419-23.

95. Mohapatra PK, Khan AM, Prakash A, Mahanta J, Srivastava VK. Effect of arteether α/β on uncomplicated falciparum malaria cases in upper Assam. *Indian J Med Res* 1996;**104**:284-7.

96. Misra SP, Nandi J, Shiv Lal S. Chloroquine versus amodiaquine in the treatment of *Plasmodium falciparum* malaria in northeast India. *Indian J Med Res* 1995;**102**:119-23.

97. Srivastava HC, Sharma SK, Bhatt RM, Sharma VP. Studies on *Plasmodium vivax* relapse pattern in kheda district, Gujarat. *Indian J Malariol* 1996;**33**:173-9.

98. Garg MR, Gogtay NJ, Kshirsagar NA. Efficacy of sulfadoxine - pyrimethamine in chloroquine resistant falciparium malaria in Bombay. *J Assoc Physic India* 1996; **44**:683-5.

99. Solomon S, Kurian N, Ramadas P, Rao PS. Incidence of nerve damage in leprosy patients treated with MDT. *Inter J Lep Mycobac Dis* 1998;**66**:451-6.

100. Zaheer SA, Beena KR, Kar HK, Sharma AK, Misra RS, Mukherjee A, *et al.* Addition of immunotherapy with mycobacterium ω vaccine to multi-drug therapy benefits in multibacillary leprosy patients. *Vaccine* 1995;**13**:1102-10.

101. Katoch K, Katoch VM, Natrajan M, Bhatia AS, Sreevatsa *et al.* Treatment of bacilliferous BL/LL cases with combined chemotherapy and immunotherapy. *Inter J Lep Mycobac Dis* 1995;**63**:202-12.

102. Rao PS, Ramachandran A, Sekar B, Ravi S, Subramanian M. Ofloxacin-containing combined drug regimens in the treatment of lepromatous leprosy. *Lep Rev* 1994;**65**:181-9.

103. Arunthathi S, Satheesh KK. Does Clofazimine have a prophylactic role against neuritis?. *Lep Rev* 1997;**68**:233-41.

104. Ebenezer GJ, Suneetha S, Arunthathi S. Clinical and histopathological activity in paucibacillary leprosy patients after fixed-duration multidrug therapy. *Lep Rev* 1997; **68**:218-24.

105. Husain S, Mishra B, Malaviya GN. Rifampicin and isoniazid in the treatment of leprous nerve abscesses. *Acta Leprol* 1997;**10**:147-50.

106. Chaudhury S, Hajra SK, Mukerjee A, Saha B, Majumdar V *et al.* Immunotherapy of lepromin-negative borderline leprosy patients with low-dose convit vaccine as an adjunct to multidrug therapy: a six-year follow-up study in Calcutta. *Inter J Lep Mycob Dis* 1997;**65**:56-62.

107. Majumder V, Mukerjee A, Hajra SK, Saha B, Saha K. Immunotherapy of far-advanced lepromatous leprosy patients with low-dose convit vaccine along with multidrug therapy (Calcutta trial). *Inter J Lep Mycob Dis* 1996;**64**:26-36.

108. Job CK, Jayakumar J, Aschhoff M, Mathan MM. Viability of mycobacterium leprae in skin and peripheral nerves and persistence of nerve destruction in multibacillary patients after 2 years of multidrug therapy. *Inter J Lep Mycob Dis* 1996;**64**:44-50.

109. Vijayakumaran P, Jesudasan K, Manimozhi N. Fixed-duration therapy (FDT) in multibacillary leprosy; efficacy and complications. *Inter J Lep Mycob Dis* 1996;**64**:123-7.

110. Jesudasan K, Vijayakumaran P, Manimozhi N, Rao PS, Samuel P. Effectiveness of MDT in multibacillary leprosy. *Inter J Lep Mycob Dis* 1996;**64**:128-32.

111. Jesudasan K, Vijayakumaran P, Manimozhi N, Jeyarajan T, Rao PS. Absence of relapse within 4 years among 34 multibacillary patients with high BIs treated for 2 years with MDT. *Inter J Lep Mycob Dis* 1996;**64**:133-5.

112. Vijayakumaran P, Manimozhi N, Jesudasan K, Arunthathi S, Jacob M, Samuel P. Leucocytopenia after rifampicin and ofloxacin therapy in leprosy. *Lep Rev* 1997;**68**:10-5.

113. Rao PS, Subramanian M, Subramanian G. Deformity incidence in leprosy patients treated with multidrug therapy. *Indian J Lep* 1994;**66**:449-54.

114. Thomas A, Hari L, Nagarajan M, Prabhakar R. Relapses during long-term follow up with drug-susceptible *M. leprae* among multibacillary leprosy patients treated with multidrug therapy regimens; case reports. *Inter J Lep Mycobac Dis* 1995;**63**:391-4.

115. Vijayakumaran P, Jesudasan K, Mozhi NM, Samuel JD. Does MDT arrest transmission of leprosy to household contacts?. *Inter J Lep Mycob Dis* 1998;**66**:125-30.

116. Darius EJ, Selvasekar A, Mani MN, Jesudasan K. Ulnar abscess: 4 months after release from control with paucibacillary-multidrug therapy. *Lep Rev* 1997;**68**:173-4.

CLINICAL PHARMACOLOGY

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1. Introduction

Clinical Pharmacology was started in India, in late 1960s. The focus was on investigation of new drugs produced by research centres in India or in developed countries. During the period 1988-93 (reviewed by Dr.R.D.Kulkarni[1]) many new centres developed in the country. Over 100 papers were published in peer reviewed journals and active work was done in pharmacokinetics and factors affecting pharmacokinetics, dosage form, drug delivery, adverse drug reaction monitoring.

In 1994-98, the Clinical Pharmacologists while continuing to carry out research in these areas contributed directly to patient care through the out patient clinics run by the departments of Clinical Pharmacology and the therapeutic drug monitoring services and ADR monitoring. They have contributed to policy making in National programs, drug selection using essential drugs list, prescribing practices through medical information service and teaching programs (continuing medical education).

2. Studies on new drugs

Synthesis and preclinical investigation of new drug molecules are carried out at only a few centres such as Central Drug Research Institute (CDRI), Lucknow, Regional Research Laboratory (RRL), Jammu, and Pharmaceutical industries such as Ranbaxy Labs Ltd.

CDRI investigated the safety and efficacy of synthetic drugs like centchroman, antimalarials, α,β arteether, 80/53, in volunteers and patients. It also standardized, characterized, purified plant products such as memory enhancers bacoside, hepatoprotective picroliv, lipid lowering gugulipid and antileprosy vaccine *M.Habana.* Centchroman a phenylchroman derivative with weak estrogenic and potent antiestrogenic activity has been investigated for breast cancer and bone loss. Lal studied bioavailability of centchroman tablets marketed by two companies and found it to be comparable[2].

α,β arteether a derivative of artemisin has been found to be safe and effective in 94% patients of *P. falciparum* malaria given three doses of 150 mg *i.m.* in Phase II and Phase III studies[3,4,5]. Anklesaria compared the effects of 80/53 with primaquine (PQ) on human erythrocytes *in vitro* and noted that 80/53 did not damage normal and G6PD deficient erythrocytes to the same extent as PQ[6].

Bacosides (A & B) obtained from *Bacopa Monniera* were found to enhance memory in animal models. It was well tolerated in single doses and multiple doses in Phase I studies in normal volunteers[7]. Picroliv a standardized fraction of root and rhizome of *Picrorhiza kurroa* has been investigated as a hepatoprotective agent. A study on proliferative T cell response to mycobacterial PPD, in subjects with tuberculoid leprosy and endemic normal, was carried out. Picroliv augmented T cell response suggesting that it could be useful as an adjunct to chemotherapy or as a short term prophylactic agent[8]. *Pipali Rasayana* prepared from Palash (*Butea monosperma lamk Kutze*, legumiriaceae) and Pippali (*Piper longum L*; Piperaceae) given for 15 days, 1 gm orally, cleared giardiasis in 23/25 patients[9].

At Mumbai, safety of amocarzine a new macrofilaricidal drug (WHO) was investigated in a Phase I study in 44 normal healthy volunteers. The drug was also investigated in Ghana for effect in onchocerciasis patients. It was noted that in Indian volunteers it produced CNS side effects at lower doses than reported in patients in Ghana[10].

New drug delivery systems were developed by pharmaceutical industry and also academic pharmacy departments. Liposomal drug delivery systems were developed by CDRI (Lucknow), PGIMER, (Chandigarh), AIIMS (New Delhi) and other centres. At the Seth G.S.Medical College and KEM Hospital, with funding from Department of Biotechnology, liposomal amphotericin was developed and investigated in Phase I, II and III studies in patients of systemic fungal infection and leishmaniasis. In leishmaniasis it was found to be safer than amphotericin, duration of treatment was shorter. It was found to be effective in resistant cases. This drug is to be marketed shortly[11-14].

3. Studies with marketed drugs

3.1. National Program drugs

During this period, resurgence of infectious diseases like malaria and problems in treatment of tuberculosis were the issues of great importance.

Antimalarial drugs were being used haphazardly by private doctors and in inadequate doses by the public health department[15]. Potkar *et al* [16] showed that 10 mg/kg dose of antimalarials used by the public health department as per National malaria eradication program recommendation (NMEP) was ineffective in 15% of *P.falciparum* patients while full dose of 25 mg/kg was ineffective in 5% cases.

It was noted that 25% patients failed to respond to second line of drug, sulphadoxine pyrimethamine[17]. Escalation of resistance to chloroquine was noted by Garg *et al.* Thus from 15% in 1993, it increased to 60% in 1997. It was hypothesized that drug resistance which arose partly due to sub-therapeutic doses being used in the population, could have contributed to the resurgence of malaria in the city. *P.vivax* strains were known to be fully sensitive to chloroquine till then however chloroquine resistant *P.vivax* was detected for the first time in India and reported by Garg *et al* [18]. Gogtay *et al* [19] reported the incidence to be 2% in Mumbai. Similarly nonresponse to antirelapse effect of primaquine was also noted[20].

Roy[21] investigated pharmacokinetics and clinical efficacy of 2 doses (10 and 5 mg/kg) of isoniazid in children with pulmonary tuberculosis, in a randomized open controlled clinical trial. All patients received rifampicin (10 mg/kg) as well as pyrazinamide (30 mg/kg). At 6 month follow up both groups had comparable clinical and radiological improvement and levels were above MIC at 6 and 24 hr in both groups. Mahajan[22] studied rifampicin levels with 10 mg and 7.5 mg/kg in 10 children, each, of tuberculous meningitis and found the levels to be above MIC with both doses.

Anand reported appearance of new cysticercus nodules after treatment with albendazole[23].

3.2. CNS drugs

Kulkarni *et al*[24] carried out double blind placebo controlled Phase II study of sumatriptan in 23 patients of acute migraine. Sumatriptan reduced headache and all associated symptoms. Drowsiness and sleep was seen in some patients but no unexpected or severe adverse effect was seen.

Usha Rani *et al*[25] in a randomized double blind, parallel study compared fluoxetine, amitriptyline and placebo in patients with chronic rheumatic pain. At the end of 4 weeks treatment fluoxetine and amitriptyline significantly reduced pain intensity compared to placebo, fluoxetine being superior and better tolerated. Garg *et al* [26] and Nagarkatti [27] *et al* evaluated flurbiprofen granules and piroxicam dispersible tablets in the management postoperative and post episiotomy pain in a prospective, single blind, parallel group study. Flurbiprofen granules had faster onset of analgesia, higher SPID and TOTPAR scores and was better tolerated.

Khajuria [28] investigated the effect of diazepam, propranolol and alcohol given singly or in combination on simple reaction time, multiple choice reaction time, critical flicker fusion frequency, digit cancellation test and noted an additive effect (impairment) on cnitical flicker fusion frequency with alcohol and propranolol.

Sunil Kumar compared effect of ketorolac with nefopam in post operative pain following laparotomy 30 mg ketorolac produced longer lasting analgesia compared to 20 mg nefopam[29].

Naidu *et al* investigated the effect of ketorolac and diclofenac sodium on gastroduodenal mucosa. No change was seen in ketorolac group while one patient with diclofenac had gastric and duodenal ulcer[30]. The number of patients in the study were small to detect statistical difference.

3.3. Cardiovascular Drugs

Satia [31] noted that enalapril given for 9 months effectively controlled BP; lowered serum lipids favorably and did not alter blood glucose. Gupta[32] and Chattopadhyay[33] reported deterioration of carbohydrate metabolism by nifedipine.

Balani[34] in a double blind study found short-term nitroxazepine to be superior to placebo in reducing diastolic BP in mild hypertensive patients. Satia[35] found in a controlled clinical trial of nifedipine and atenolol that while both drugs reduced BP, atenolol significantly increased triglyceride levels and decreased HDL cholesterol levels. Patki[36] studied the effect of prazosin GITS, atenolol, nifedipine SR and enalapril and found that prazosin GITS inhibited ADP induced platelet aggregation, in uncomplicated hypertension. It was a randomized, observer blind, parallel group comparative study where the drug was given after 14 days of placebo period. No reflex tachycardia or first dose effect was seen with any drug.

Yegnanarayan[37] studied the effect of Abana and propranolol on left ventricular hypertrophy in hypertensive patient using echocardiography. Abana and propranolol were found to reduce cardiac mass. Abana probably causes down regulation of β receptors.

Adhikari compared safety and efficacy of lovastatin with gemfibrozil and found that it was superior in lowering LDL cholesterol and raising HDL cholesterol and is better tolerated[38]. Usha Rani evaluated clinical efficacy and safety of hydroxyethyl starch[39].

Nayak *et al* studied[40] the effect of hypertension and antihypertensive agents on hemostatic variables and concluded that there is a need to monitor them. Desai studied platelet aggregation and found that nifedipine (10 mg) and aspirin (75 mg) inhibited platelet aggregation in hypertensive patients and normal volunteers[41].

3.4. Miscellaneous

Shahani *et al* in a randomized open controlled study evaluated biorelease of fluticasone, a topically active synthetic corticosteroid and found that it was effective in reducing histamine wheal and flare response and that antibacterials and antifungals did not alter its biorelease[42].

Rajaram *et al* compared the effectiveness of cetirizine and astemizole in a double blind randomized trial in perennial allergic rhinitis and found them to be comparable[43].

Dharmadhikari *et al* [44] compared in a double blind placebo controlled study the effect of newer antihistaminics astemizole, terfenadine and cetirizine with older antihistaminics and placebo in 6 healthy male and female volunteers, on salivary flow. Most antihistaminics except newer ones reduced salivary flow.

Minocha noted that addition of H_2 antagonist cimetidine, to H_1 antagonist therapy, produced moderate to good improvement of various parameters of urticaria in 85% patients[45].

Kshirsagar and Gupta [46] compared the effect of combined administration of Ibuprofen and pseudoephedrine hydrochloride and pseudoephedrine alone on common cold in a double blind randomized comparative study.

Using ultrasonography and uroflowmetry in addition to clinical examination, Basalingappa investigated the effectiveness of prazosin in the treatment of benign prostatic hyperplasia. They noted that prazosin was effective at doses that produced minimal adverse effects related to vasodilator properties[47].

Rama Raju evaluated use of fluoxetine in premature ejaculation using "Inventory at sexual satisfaction" (GRISS) questionnaire[48].

Pandey *et al* [49] investigated the status of free radicals and their scavenging enzymes in pregnancy induced hypertension. Gujrati *et al*[50] studied placental nuclear monoamine oxidase as biochemical and histochemical evidence for hyperserotonic state in preclampsia and eclampsia.

Rajwade *et al* [51] noted that phospholipids and arachidonic acid were significantly altered in patients with acute stroke.

4. Pharmacodynamic and Pharmacokinetic methods in Clinical Pharmacology

4.1. Pharmacodynamic

Classical methods for assessing the effects of CNS active drugs such as reaction time, critical flicker fusion frequency and cardiovascular drugs such as blood pressure and platelet function, continued to be used. Newer methods have been developed and standardized for investigating the effects of chemotherapeutic agents, immunomodulators, selective α receptor blockers used for prostate hyperplasia etc.

Sleep polysomnography technique is being used to assess effects of traditional methods. Gitanjali[52] compared effect Nilambari Raga and Kalyani Raga, Nilambari Raga is supposed to have sleep inducing and promoting qualities. No difference in sleep architecture or feeling of quality of sleep was found. Usha Rani[53] evaluated the effect of a polyherbal preparation on patients with chronic primary insomnia and found improvement in various sleep parameters using polysomnograph and subjective symptoms.

In vitro tests to detect drug sensitivity of *P.falciparum* was developed. Biswas *et al*[54] used non radioactive thymidine analogue bromodeoxyuridine to detect growth and found antimalarial activity of monoterpinic fragment analogs of aplasmomycin[55].

Several immunodiagnostic tests for infectious diseases are now marketed. Application of Parasight F test to diagnose drug resistance was investigated by Vakharia[56]. Parasight F test remained positive days after disappearance of parasitemia and hence would be falsely positive in patients with parasites sensitive to antimalarials. Valecha *et al* found sensitivity and specificity of commercial immunochromato-graphic test (ICT) to be 98.6% and 97% respectively and also noted positivity after curative chemotherapy[57].

4.2. Pharmacokinetic

Method for measuring plasma concentrations have been developed. Sekkar *et al* [58] developed a rapid reproducible sensitive HPLC method for terazosin while Ravi[59] reported a method for secnidazole. Sulochana developed a HPLC method for ampicillin and sulbactam[60].

Paliwal and Gupta[61] set up and validated a HPLC assay with fluorescence detection for new compound 80/53, a safer and less toxic analogue of primaquine. Pharmacokinetic of centchroman in serum and milk of nursing mother was investigated using HPLC assay. Centchroman level in breast milk was low and unlikely to be of any physiological consequence[62]. In healthy female subjects, the absorption and disposition of centchroman was found to be first order, reproducible and dose dependent[63].

Biswas *et al* studied pharmacokinetic profile of intraarticular lignocaine during knee joint arthroscopy[64].

4.3. Mechanism of action

Kumar *et al* [65] have been conducting studies on receptor transcripts for androgen, epidermal growth factor and basic fibroblast growth factor in postmortem prostate tissue.

Goel[66] studied prostaglandin release by human colonic mucosal incubates and noted a concentration dependent stimulatory effect of bergenin and nonbergenin, suggesting that gastroprotective effect, could be due to increased prostaglandin production.

Joshi *et al* [67] investigated the possible mechanism involved in altered digoxin levels in diabetic patients. Anaokar *et al* [68] studied the effect of cardiopulmonary bypass on plasma digoxin levels. Joglekar *et al* [69] studied digoxin concentration in Indian patients before cardiothoracic surgery and noted that despite adequate dosing, plasma concentration of digoxin were below therapeutic range.

5. Monitoring and prevention of ADR

ADR monitoring in India is important due to different prescribing practices. Ciprofloxacin, reported to cause arthropathy in animals, is contraindicated in children. But it is used as a life saving drug for children with resistant Salmonella infection. Karande and Kshirsagar[70] carried out postal survey to investigate acute and long term effects of ciprofloxacin in children and found acute arthropathy but no long term or delayed effect.

Mefloquine induced grand mal seizure in a patient was reported[71]. The patient had previous history of epilepsy, but did not reveal it due social stigma, bringing out special circumstances of India. The same group also noted seizures in patients receiving ciprofloxacin and mefloquine (both epileptogenic) combination prescribed to cover Salmonella and malaria in febrile patients where diagnosis cannot be confirmed due to poor facilities.

Dhume's group[72] in Goa have investigated ADRs in hospitalized patients, specially to NSAID. Unusual ADRs have been reported *e.g.,* craving for sweets following gestrinone[73] therapy famotidine (structural similarity to sulpha) induced rash in sulphonamide sensitive patient and familial sulpha sensitivity[74,75], ticlopidine induced agranulocytosis[76], tachycardia due to cisapride[77] and ciprofloxacin induced bradycardia[78] were reported.

Thomas studied ADR to antitubercular drugs[79], Taneja *et al* [80] studied Stevens Johnson syndrome. Puri reported that chronic treatment with lithium increased ADH and urine volume[81].

Kumaravel used FISH (Fluorescence In situ Hybridization) technique against monosomy 7 and 5 q in early detection of therapy related acute myeloid leukaemia (t -AML) in patients receiving cytotoxic chemotherapy. These aberrations preceded the development of clinical leukaemia by at least six months. Molecular cytogenetic techniques may be useful to clinical pharmacologists in early detection of t-AML[82].

6. Rational drug therapy

Considerable work has been done in the area of studying prescribing practices in hospital and general practice.

Studies by Ansari [83], in Allahabad, Muralikrishnan in Vishakhapatnam and Kshirsagar in Pune[84], have elucidated the prescribing trends in General practice. Fixed drug combination dominated prescribing, less than 60% of drugs were from the essential drugs list and more than 30% prescriptions were found to be irrational.

Beri [85] studied the use of aspirin by General practitioners in patients of suspected myocardial infarction. Despite the recommendations (*e.g.,* British Heart foundation group) for early use of aspirin, less than 10% doctors prescribed aspirin in patients suspected of myocardial infarction.

Garg *et al*[15] studied the prescribing patterns of antimalarial drugs in Mumbai and found irrationality in the choice and dose of drug. Chloroquine dose used was from 2-20 tablets and the drugs like primaquine, quinolones were used for resistant malaria.

Shewade analyzed prescribing habits in Pondicherry and found that general practitioners prescribe expensive, harmful or ineffective drugs as also injectables. Prescription from Govt. hospitals were more rational[86].

Antibiotics form a major part of prescriptions in terms of proportion of total prescriptions as well as cost. Antibiotic use specially in surgical practice has been investigated[87-89]. Inappropriateness in timing and duration of administration of antibacterial for prophylaxis of surgical infection was noted. Sood *et al* noted that 93% of drugs were prescribed under brand names. Thomas *et al*[88] while comparing practices in primary and tertiary care hospital noted that primary care centre started antibiotics after completion of procedure while tertiary care centre gave prolonged postoperative courses. Nosocomial infection rate was higher amongst these patients compared to those who received antibiotics for less than 72 hrs. Antibiotics were started empirically in 78% of tertiary hospital care cases and 100% cases in the primary hospital. Kulkarni *et al* [89] found that the cost of antibiotics could be brought down by half by rational prescribing. Gupta carried out pharmacy based auditing of antimicrobial use in tertiary care hospital[90].

In an effort to bring about change in prescribing practice, Thomas *et al* have been conducting workshops for promoting rational use. They noted that improvements seen after workshops tend to diminish if there is no reinforcement, thus emphasizing the need for regular workshops[91,92].

Natu *et al*[93] conducted a workshop for interns covering various aspects of rational drug use; Evaluation of workshop revealed that it was able to bring about attitudenal change regarding rational drug use.

Kohli *et al* [94] audited 210 prescriptions selected by students using a group discussion technique. It was observed that inadequate treatment was prescribed to patients suffering from common diseases like amoebiasis, tuberculosis and typhoid fever. Indiscriminate use of antibiotics antihistaminics, NSAIDS, vitamins, hematinics was noted.

Consensus on prescribing policies, inter-department coordination, Hospital Committees cooperation, CMES and refresher courses are ways of improving prescribing practices and should be implemented[95].

7. Bioavailability studies

Bioequivalence testing was made mandatory in India since 1988.

Bioequivalence of Indian formulations were found to be comparable with international brands of sumatriptan[96] and slow release diltiazem formulation[97].

Sachadeva studied cyclosporin bioavailability of two brands and found it to be comparable[98]. It was noted by Joseph *et al* [99] and Seth *et al* [100,101] that Indian formulations are equally bioavailable as the foreign brand but that plasma levels achieved in Indian volunteers with foreign brand of omeprazole, felodipine, norfloxacin and ciprofloxacin were higher than levels achieved with same doses in Western population. Such differences could have arisen due to low body weight of Indian population, variations in drug metabolism excretion or differences in methodology. Cross continental studies with sample analysis in same laboratory would be useful to elucidate such interpopulation differences.

In one study, plasma levels of amocarzine were found to be higher in Indian volunteers from Bombay than in patients from Ghana. HPLC analysis of samples from both populations was done in Bombay, thus removing assay variability. The same mg/kg dose was given thus removing body weight as confounding variable[10].

8. Therapeutic drug monitoring

Therapeutic drug monitoring is an important component of the Clinical Pharmacology service to patients. Rataboli *et al* [102] reported a case of recessive dystrophic epidermolysis bullosa, who was given diphenylhydantoin as a first line drug. A good correlation was found between clinical response and serum phenytoin concentration. Optimum concentration required for healing of lesions was around 7 µg/ml.

Rataboli analyzed data of 75 epileptic patients receiving phenytoin (PHT), phenobarbitone (PBT) and carbamazepine (CBZ)[103]. The highest incidence of ADRs and least satisfactory response was seen in patients receiving all three drugs. While PHT and CBZ combination had least incidence of ADR and good clinical response.

TDM Service is surprisingly relatively less used by the paediatricians. In one study several paediatric patients of epilepsy were found to have levels below the therapeutic range[104].

9. Drug interactions

A few workers have carried out drug interaction studies. Rao *et al*[105] studied the effect of single oral dose of sumatriptan on paracetamol pharmacokinetics in normal healthy volunteers and found that sumatriptan decreased C_{max} and increased T_{max} of paracetamol. Suryakumar *et al* showed[106] that rifampicin 450 mg for 6 days reduced bioavailability of diclofenac. Garg *et al*[107] investigated effect of grapefruit juice on bioavailability of carbamazepine in patients with epilepsy. Grapefruit juice resulted in significantly high peak, trough concentration and AUC, probably by inhibiting CYP3A4 enzyme in gutwall and liver.

Kulkarni *et al*[108] studied interaction of aminophylline (AMP) with single dose sodium valporate (SV) and carbamazepine (CBZ) in normal healthy volunteers. AMP significantly decreased the plasma concentration, AUC and prolonged T_{max} and $t_{1/2}$ of CBZ but not of SV.

Vaz *et al*[109] evaluated effect of caffeine (300 mg) in three divided doses on pharmacokinetics of SV and CBZ given as single dose. A significant reduction in plasma concentration and AUC of CBZ and prolongation of $t_{1/2}$ was noted.

Curcumin has poor bioavailability. Piperine is a known inhibitor of hepatic and intestinal glucuronidation. Concomitant administration of 20 mg piperine increased plasma concentration and bioavailability of curcumin[110].

10. Pharmacoeconomics

Pharmacoeconomics is analysis of costs of drug therapy to healthcare systems and society and it involves assessing the outcomes (projected) and implications to costs of pharmaceuticals products.

Roy surveyed the variation in prices of drugs used in the management of cardiovascular diseases and found wide variation and noted the urgent need to provide information to physician, on cost, bioequivalence and quality[111].

11. Conclusion

During the 1994-1998 period, Clinical Pharmacology has contributed to development of new drugs and rational use of drugs through the various research, teaching and service activities undertaken. International collaboration has benefited the growth of discipline in the country. It is expected that in the next five years this trend will continue and further use of techniques of molecular biology will enrich the discipline.

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REFERENCES

1. Kulkarni RD. Clinical Pharmacology In: Chauhan CK, editor. Pharmacology update: Reviews of research in Pharmacology in India (1988-1993), Mumbai, L.T.M. Medical College.

2. Lal J, Nityanand S, Asthana OP, Gupta RC. Comparative bioavailability of two commercial centchroman tablets in healthy female subjects. *Indian J Pharmacol* 1996; **28**:32-4.

3. Mishra SK, Asthana OP, Mohanty S, Patnaik JK, Das BS *et al.* Effectiveness of alpha, beta- arteether in acute falciparum malaria. *Trans Royal Soc Trop Med Hyg* 1995;**89**: 299-301.

4. Singh N, Shukla MM, Asthana OP, Sharma VP. Effectiveness of alpha-beta arteether in clearing *Plasmodium falciparum* parasitemia in central India (Madhya Pradesh). *Southeast Asian J Trop Med Public Health* 1998; **29**:225-7.

5. Valecha N, Sharma VP, Devi CU. A rapid immunochromatographic test (ICT) for diagnosis of *Plasmodium falciparum. Diag Micro Inf Dis* 1998;**30**:257-60.

6. Anklesaria PS, Ashar VJ, Kshirsagar NA, Gupta KC. Comparison of the effect of compound CDRI 80/53 [N-(3-acetyl-4,5-dihydro-2-furanyl)-N4-(6-methoxy-8-quinolinyl)1,4-pentanediamine] with primaquine on human erythrocytes *in vitro.* In: Kumar S, Sen SK, Dutta GP, Sharma RN, editors. Tropical diseases - Molecular biology and control strategies. 1st Ed. New Delhi. Publications & Information Directorate. 1994:256-261.

7. Singh HK, Dhawan BN. Neuropsychopharmacological effects of the Ayurvedic nootropic Bacopa monniera linn (Brahmi). *Indian J Pharmacol* 1997;**29**:S359-S365.

8. Sinha S, Mehrotra J, Bala L, Jaiswal AK, Dhawan BN. Picroliv the iridoid glycoside fraction of *Picrorhiza kurroa,* selectively augments human T cell response to mycobacterial protein antigens. *Immunopharmacol Immunotoxicol* 1998;**20**:579-88.

9. Agarwal AK, Tripathi DM, Sahai R, Gupta N, Saxena RP *et al.* Management of giardiasis by a herbal drug 'Pippali Rasayana': a clinical study. *J Ethno Pharmacol* 1997; **56**:233-6.

10. Awadzi K, Apokey NO, Attaly SK, Addy ET, Duke BOL *et al.* The safety and efficacy of amocarzine in African onchocerciasis and the influence of ivermectin on the clinical and parasitiology response to treatment. *Ann Trop Med Parasitol* 1997;**91**: 281-96.

11. Kshirsagar NA, Bodhe PV, Kotwani RN. Targeted drug delivery in visceral leishmaniasis. *J Parasitic Dis* 1997;**21**: 21-4.

12. Karande SC, John Boby KF, Lahiri KR, Jain MK, Kshirsagar NA *et al.* Successful treatment of antimony-resistant visceral leishmaniasis with liposomal amphotericin B in a child. *Trop Doc* 1995;**25**:80-81.

13. Bodhe PV, Pathare AV, Kshirsagar NA, Pandya SK. Treatment of visceral leishmaniasis is with a 10 days course of L-AMP-LRC (Bombay) A liposomal AmpB. *J Ass Phy India* 1996;**44**:222.

14. Kotwani RN, Gokhale PC, Kshirsagar NA, Pandya SK. Optimizing dosage regimens of liposomal amphotericin B using aspergillus murine model. *Indian J Pharmacol* 1996; **28**:88-92.

15. Garg MR, Nagnur S, Potkar CN, Kshirsagar NA. General practitioners and treatment of malaria-need for continuing medical education. *J Assoc Physicians India* 1995;**43**: 584-5.

16. Potkar CN, Kshirsagar NA, Kathuria R. Resurgence of malaria and drug resistance in *Plasmodium falciparum* and *Plasmodium vivax* species in Bombay. *J Assoc Physicians India* 1995;**43**:336-8.

17. Garg MR, Gogtay NJ, Kshirsagar NA. Efficacy of sulfadoxine pyrimethamine in chloroquine resistant falciparum malaria in Bombay. *J Assoc Physicians India* 1996;**44**:683-5.

18. Garg MR, Gopinathan N, Bodhe P, Kshirsagar NA. Vivax malaria resistant to chloroquine : Case reports from Bombay. *Trans Royal Soc Trop Med Hyg* 1995;**89**:656-7.

19. Gogtay NJ, Garg MR, Bodhe PV, Tilve GH, Kshirsagar NA. Response of vivax malaria to chloroquine in Mumbai. *J Assoc Physicians India* 1998;**46**:843-4.

20. Gogtay NJ, Garg MR, Kadam V, Kamtekar K, Kshirsagar NA. Efficacy of a 5 day primaquine regimen as anti-relapse therapy for *Plasmodium vivax. Trans Royal Soc Trop Med Hygi* 1998;**92**:341.

21. Roy V, Tekur U, chopra K. Pharmacokinetics of isoniazid in pulmonary tuberculosis- a comparative study at two dose levels. *Indian Pediatrics* 1996;**33**:287-91.

22. Mahajan M, Rohatgi D, Talwar V, Patni SK, Mahajan P, Agarwal DS. Serum and cerebrospinal fluid concentrations of rifampicin at two dose levels in children with tuberculous meningitis. *J Commun Dis* 1997;**29**:269-74.

23. Anand KS, Abraham J, Gowda S, Ratnakar C. Apperance of new cysticercosis nodules with albendazole therapy. *Indian J Pharmacol* 1994; **26**:165.

24. Kulkarni SD, Pandit VA, Dange SV, Beri SG, Mitra DK. Phase II study of sumatriptan in Migraine 1997;**1**.

25. Usha Rani P, Naidu MUR, Ramesh T, Shobha U, Vijay T, Prasad VBN. A comparative study of the effects of fluoxetine, amitriptyline and placebo on salivary flow and haemodynamic parameters in patients with chronic pain. *Asia Pac J Pharmacol* 1995;**10**:21-4.

26. Garg MR, Gopinathan N, Kantharia CV, Dalvi AN, Divekar A *et al.* A comparative evaluation of flurbiprofen effervescent granules and piroxicam dispersible tablets in the management of post-operative pain. *J Gen Med* 1996; **9**:43-8.

27. Nagarkatti D, Parulekar S, Thatte U, Dahanukar SA. A comparative evalution of flurbiprofen granules (Froben FR) and piroxicam dispersible tablets in the management of post-episiotomy pain. *Indian Pract* 1995;**48**:1167-74.

28. Khajuria V, Kapoor B, Raina RK. Studies on psychomotor performance in healthy volunteers after diazepam, propranolol and alcohol given alone or in combination. *Indian J Physiol Pharmacol* 1995;**39**:242-6.

29. Sunil Kumar, Manoj Kumar SP, Gupta RL. Ketorolac vs nefopam :Comparison of single intravenous dose in relieving post-operative pain following laparotomy. *Indian J Pharmacol* 1996;**28**:240-3.

30. Naidu MUR, Reddy DN, Shobha JC, Prasad VBN, Ramesh Kumar T. Endoscopic evaluation of the effects of ketorolac and diclofenac sodium on the gastroduodenal mucosa. *Indian J Pharmacol* 1996;**28**:194-5.

31. Satia MC, Shukla ML, Goyal RK. Effects of enalapril on lipid profile in diabetic and non-diabetic essential hypertensive patients. *Indian J Physiol Pharmacol* 1996; **40**:83-6.

32. Gupta N, Ansari KU. Deterioration of carbohydrate metabolism by nifedipine. *Indian J Med Sci* 1996;**50**:201-3.

33. Chattopadhyay RN, Roy RK, Mandal S, Lahiri HL, Maitra SK *et al.* Effect of nifedipine on glucose tolerance in normal volunteers and patients with diabetes mellitus. *Indian J Pharmacol* 1995;**27**:34-6.

34. Balani ND, Parhate SM, Thawani VR, Deshpande AM. Effects of nitroxazepine on diastolic blood pressure in mild hypertensive patients - a short term clinical study. *Indian J Physiol Pharmacol* 1995;**39**:293-5.

35. Satia MC, Shukla ML, Goyal RK. Comparative effects of atenolol versus nifedipine on serum lipids and other biochemical parameters in diabetic and non-diabetic hypertensive subjects. *Indian J Physiol Pharmacol* 1995;**39**:237-41.

36. Patki KC, Joglekar SJ, Kamat SK, Thatte UM, Yeolekar ME *et al.* Effect of prazosin GITS, atenolol, nifedipine SR, and enalapril on ADP-induced platelet aggregation. *J Assoc Physicians India* 1998;**46**:26-29.

37. Yegnanarayan R, Sangle SA, Sirsikar SS, Mitra DK. Regression of cardiac hypertrophy in hypertensive patients - comparison of Abana with propranolol. *Phytother Res* 1997; **2**:257-9.

38. Adhikari Prabha MR, Kannampilly J, Hegde BM, Pereira P, Dungath M, Chowta N. A comparative double blind randomised study of gemfibrozil and lovastatin in hyperchlesterolemia. *Indian J Pharmacol* 1997;**29**:314-17.

39. Usha Rani P, Naidu MUR, Manimala Rao S, Murthy T. VSSN, Ramesh Kumar T. Evaluation of clinical efficacy and safety of hydroxyethyl starch. *Indian J Pharmacol* 1996; **28**:181-4.

40. Nayak VK, Desai NK, Gupta KC. Effect of hypertension and antihypertensive agents on haemostatic variables. *Indian J Pharmacol* 1997;**29**:86-91.

41. Desai AA, Nayak VK, Desai NK, Gupta KC. Effect of nifedipine and aspirin on platelet aggregation: A study in hypertensive patients and normal volunteers. *Indian J Pharmacol* 1995;**27**:167-70.

42. Sahani SR, Kerajani HR, Sharma K, Cooverji ND. Anti-inflammatory effects of fluticasone, a topically active syntheitc corticosteroid and its combination formulations in histamine induced cutaneous responses in healthy human volunteers. *Indian J Pharmacol* 1998;**30**:245-9.

43. Rajaram S, Suthakaran C, Pradhan SC, Majumder NK, Bapna JS. Double blind randomized clinical trial of cetirizine in the treatment of perennial altergic rhinitis. *Indian J Pharmacology* 1994;**26**:112-6.

44. Dharmadhikari SD, Shrivastava MP. Effects of oxyphenonium bromide and some antihistaminics salivary flow in human volunteers. *Indian J Physiol Pharmacol* 1997;**41**: 183-4.

45. Minocha YC, Minocha KB, Sood VK, Dogra A. Evaluation of H_2-receptor antagonists in chronic idiopathic urticaria. *Indian J Dermatol Venereol Leprol* 1995;**61**:265-7.

46. Kshirsagar NA, Gupta KC. Comparison of Arinac and pseudoephedrine in common cold. *Indian Pract* 1994;**47**.

47. Basalingappa S, Ignatius J, Madappa KM. Clinical evaluation of prazosin in symptomatic benign prostatic hyperplasia-an Indian experience. *Indian J Pharmac* 1997;**29**: 420-5.

48. Rama Raju GA, Usha Rani, Naidu MUR, RameshT, Shobha JC. Evaluation of fluoxetine in premature ejaculation. *Indian J Pharmacol* 1997;**29**:204-5.

49. Pandey S, Gujrati VR, Chandravati, Sanger KC, Shanker K. Status of free radicals and their scavenging enzymes in pregnancy induced hypertension. *Bollettino chimico Farmaceutico* 1996;**135**:472-6.

50. Gujrati VR, Shanker K, Vrat S, Chandravati, Parmar SS. Novel appearance of placental nuclear monoamine oxidase:biochemical and histochemical evidence for hyperserotonomic state in preeclampsia-eclampsia. *Am J Obst Gynecol* 1996;**175**:1543-50.

51. Rajwade NA, Desai NK, Gupta KC. Evaluation of serum lipids in patients of acute stroke. *J Assoc Physicians India* 1996;**44**: 544-5.

52. Gitanjali B. Effect of the karnatic music raga "Neelambari" on sleep architecture. *Indian J Physiol Pharmacol* 1998; **42**:119-22.

53. Usha Rani P, Naidu MUR. Subjective and polysomnographic evaluation of a new herbal preparation in insomnia. Phytomed 1998 (In press).

54. Biswas S, Valecha N. Kundu ML, Balu N, Thomas JV, Bhat SV. *In vitro* antimalarial activity of monoterpenic fragment analogues of aplasmomycin. *Indian J Exptl Bio* 1995; **33**:521-3.

55. Biswas S, Valecha N. Bromo-deoxyuridine based assay for detection of parasite and drug sensitivity in *Plasmodium falciparum in vitro. Indian J Exptl Bio* 1996; **34**:1237-40.

56. Vakharia S, Gopinathan N, Kshirsagar NA. The parasight F test for detection treatment failure. *Trans Royal Soc Trop Med Hyg* 1997;**91**:490-1.

57. Valecha N, Sharma VP, Devi CU. A rapid immunochromatographic test (ICT) for diagnosis of *Plasmodium falciparum. Diag Microbiol Inf Dis* 1998;**30**:257-60.

58. Sekhar EC, Rao TR, Sekhar KR, Naidu MU, Shobha JC *et al.* Determination of terazocin in human plasma using high-performance liquid chromatography with fluorescence detection. *J Chromat Biomed Appl* 1998;**710**:137-42.

59. Ravi SK, Naidu MU, Sekhar EC, Rao TR, Shobha JC *et al.* Rapid and selective analysis of secnidazole in human plasma using high performance liquid chromatography with ultraviolet detection. *J Chromat Biomed Appl* 1997;**691**:208-11.

60. Sulochana KN, Bhooma V, Madhavan HN, Ramakrishnan S, Biswas A. High performance liquid chromatographic method for simultaneous determination of ampicillin and sulbactam in biological samples. *Indian J Pharmacol* 1995; **27**:189-92.

61. Paliwal JK, Gupta RC. A rapid and sensitive high performance liquid chromatographic assay of the new antimalrial compound 80/53 in serum with a novel sample clean-up method and its pharmacokinetics in rabbits. *J Pharm Biomed Anal* 1998;**17**:775-83.

62. Gupta RC, Paliwal JK, Nityanand S, Asthana OP, Lal J. Centchroman: a new non-steroidal oral contraceptive in human milk. *Contraception* 1995;**52**:301-5.

63. Lal J, Asthana OP, Nityanand S, Gupta RC. Pharmacokinetics of centchroman in healthy female subjects after oral administration. *Contraception* 1995;**52**:297-300.

64. Biswas NR, Mandal NG, Lalit Kumar PGL. Pharmacokinetic profile of intra-articular lignocaine during knee joint arthroscopy. *Indian J Pharmacol* 1995;**27**:193-4.

65. Kumar VL, Majumder PK, Murty OP, Kumar V. Detection of receptor transcripts for androgen, epidermal growth factor and basic fibroblast growth factor in human prostate postmortem. *Inter Urol Nephrol* 1998;**30**:301-4.

66. Goel RK, Maiti RN, Manickam M, Ray AB. Antiulcer activity of naturally occurring pyrano-coumarin and isocoumarins and their effect on prostanoid synthesis using human colonic mucosa. *Indian J Exptl Bio* 1997;**35**:1080-3.

67. Joshi SD, Santani DD, Sheth JJ, Mehta HC, Dave KC, Goyal RK. Investigation into the possible mechanisms involved in altered digoxin levels in diabetic patients. *Indian J Physiol Pharmacol* 1996;**40**:65-9.

68. Anaokar SM, Thorat SP, Tendolkar AG, Mukadam ME, Dahanukar SA, Thatte UM. Cardiac surgery and plasma digoxin levels. *J Assoc Physicians India* 1996;**44**:694-6.

69. Joglekar SJ, Thatte UM, Anand S, Dahanukar SA, Tendolkar AG. Digoxin concentration in Indian patients. *Lancet* 1997;**349**:1326-7.

70. Karande SC, Kshirsagar NA. Ciprofloxacin use : Acute arthropathy and long -term follow up. *Indian Ped* 1996;**33**: 910-6.

71. Garg MR, Gopinathan N, Kshirsagar NA. Mefloquine induced grandmal seizures in a case of uncomplicated falciparum malaria. *J Assoc Physicians India* 1996;**44**:750.

72. Rataboli PV, Diniz D'souza RS, Dhume VG. Adverse drug reactions to non steroidal anti-Inflammatory drugs in hospitalized patients. *Indian J Clin Pract* 1994;**5**.

73. Rataboli PV, Kunkolienkar AS, Diniz D'souza RS, Dhume VG. An unusal craving for sweets following gestrinone therapy. *Obs Gynae Today* 1997;**11**.

74. Kiran V. Barar Rajnish Vijay Barar. Sulphonamide-like hypersensitivity reactions from some apparently safe common drugs with identical moieties. *Indian J Pharmacol* 1998;**30**:273-4.

75. Roy V, Gupta U, Sachdeva G, Bapna JS. Adverse Drug reaction to cimetidine, famotidine and omeprazole with special reference to hepatic functions and lipid profile : a prospective study. *Indian J Pharmacol* 1997;**29**:20-3.

76. Guido S, Rao SP, Singh G, Joseph T. Ticlopidine induced agranulocytosis in an Indian patient -A case report. *Indian J Pharmacol* 1995;**27**: 201.

77. Usha Rani P, Naidu MUR, Ramesh Kumar T, Shobha U, Ajit Kumar. Tachycardia during cisapride therapy. *Indian J Pharmacol* 1994;**26**:233-4.

78. Dhobi GN, Shafiqa TA. Ciprofloxacin induced bradycardia. *Indian J Pharmacol* 1994;**26**:164.

79. Thomas M, Seshadri MS, Cherian AM. A prospective study of adverse drug reactions to anti tuberculous drugs. *Pharmacoepidemiol Drug Safety* 1995;**4**:97-100.

80. Taneja A, Gupte PD, Mangalvedhekar SS, Kshirsagar NA. Steven Johnson syndrome - Results of a three year study in KEM Hospital. *Indian Pract* 1998;**51**:799-802.

81. Puri VN. Lithium induced changes in urinary antidiuretic hormone (ADH) in manic depressive psychosis. *Indian J Pharmacol* 1994;**26**:231-2.

82. Kumaravel B, Kumaravel TS, Gajendiren N, Mary NM, Mansyur Arif. Molecular genetic markers predict development of drug-induced leukemias in patients undergoing cytotoxic therapy. *Indian J Pharmacol* 1997;**29**:405-10.

83. Ansari KU, Singh S, Pandey RC. Evaluation of prescribing pattern of doctors for rational drug therapy. *Indian J Pharamcol* 1998;**30**:43-6.

84. Kshirsagar MJ, Langade D, Patil S, Patki PS. Prescribing patterns among medical practitioners in Pune, India. *Bulletin World Health Org* 1998;**76**:271-5.

85. Beri SG, Dange SV, Kulkarni SD. Survey of use of aspirin by general practitioners in patients of suspected acute myocardial infarction. *Med J Western India* 1997;**25**:6-8.

86. Shewade DG, Pradhan SC. Auditing of prescriptions in a government teaching hospital and four retail medical stores in Pondicherry. *Indian J Pharmacol* 1998;**30**:408-10.

87. Sood PR, Thatte UM, Rege NN, Dahanukar SA, Supe AN, Bapat RD. General prescribing trend in surgical inpatients of a teaching hospital. *Indian Pract* 1997;**50**:857-61.

88. Thomas M, Govil Shalini, Moses BV, Abraham Joseph. Monitoring of antibiotic use in a primary and tertiary care hospital. *J Clin Epidemiol* 1996;**49**:251-4.

89. Kulkarni C, Chalissery P, Jose J, Babba R, Joseph T. Prescribing pattern of antibiotics in abdominal surgery at a teaching hospital: A pilot study. *Indian J Clin Pharmacol Therap* 1994;**10**:4-8.

90. Gupta N, Sharma D, Garg SK, Bhargava VK. Auditing of prescriptions to study utilization of antimicrobials in a teritary hospital. *Indian J Pharmacol* 1997;**29**:411-15.

91. Thomas M, Mathai D, Cherian AM, Seshadri MS, Ganesh A, Moses P. Promoting rational drug use in India. *World Health Forum* 1995;**16**:33-5.

92. Thomas M. Educating general practitioners in India on rational drug use. *Changing Med Ed Med Prac* 1994;**5**.

93. Natu MV, Zachariah P, Zachariah A, Chand S, Singh T, Choudhary K. Rational drug use - evaluation of a training programme for interns. *J Indian Med Ass* 1995;**93**: 346-8.

94. Kohli K, Kela AK, Mehta VL. Rational drug therapy as evaluated from students clinical case reports. *Indian J Physiol Pharmacol* 1995;**39**:400-2.

95. Sood PR, Thatte UM, Rege NN, Dahanukar SA, Supe AN, Bapat RD. General prescribing trend in surgical inpatients of a teaching hospital. *Indian Pract* 1997;**50**:857-61.

96. Usha Rani P, Naidu MUR, Ramesh Kumar T, Shobha JC, Vijay T *et al.* A bioequivalence study of two brands of sumatriptan tablets. *Cur Therap Res* 1996;**57**.

97. Usha Rani P, Naidu MUR, Ramesh Kumar T, Shobha JC, Vijay T. Bioequivalence study of two slow-release diltiazem formulations using dynamic measures in healthy volunteers. *Clin Drug Invest* 1997;**14**.

98. Sachdeva A, Gulati R, Gupta U, Bapna JS. Comparative bioavailability of two brands of cyclosporin. *Indian J Pharmacol* 1997;**29**:135-7.

99. Joseph T, Gowrishankar R. Higher AUCs on oral administration of omeprazole and felodipine in Indian volunteers - are they clinically important? *Indian J Physiol Pharmacol* 1996;**40**:253-6.

100. Seth SD, Beotra A, Seth S. Comparative bioavailability of two brands of ciprofloxacin. *J Assoc Physicians India* 1995; **43**: 327-30.

101. Seth SD, Beotra A, Seth S. Comparative bioavailability of two brands of norfloxacin. *J Assoc Physicians India* 1995; **43**:324-6.

102. Rataboli PV, Naik VR, Kunkolienkar AS, Diniz D'souza RS, Dhume VG. Correlation of serum diphenylhydantoin concentrations with the clinical response in a case of recessive dystrophic epidermolysis bullosa. *Indian Pract* 1995; **48**.

103. Rataboli PV, Naik VR, Kunkolienkar AS, Diniz D'souza RS, Dhume VG. A study of serum concentrations, adverse effects and clinical response during polytherapy with diphenylhydantoin, phenobarbitone and carbamazepine in epileptic patients. *Neurosci Today* 1998;**2**.

104. Karande SC, Dalvi SS, Kshirsagar NA. Shortcomings in the pharmaco-therapy of rpileptic children in Bombay. *India J Trop Ped* 1995;**41**:247-9.

105. Rao TRK, Naidu MUR, Shobha JC, Sekhar KR, Rani PU, Sekhar EC. Effect of a single oral dose of sumatriptan on paracetamol pharmacokinetics. *Clin Drug investigation* 1995;**10**:242-6.

106. Surya Kumar J, Rao Mamidi NVS, Krishna DR, Chakrapani T. Rifampicin pretreatment reduces bioavailability of diclofenac sodium. *Indian J Pharmacol* 1995;**27**:183-5.

107. Garg SK, Kumar N, Bhargava VK, Prabhakar SK. Effect of grapefruit juice on carbamazepine bioavailability in patients with epilepsy. *Clin Pharmacol Therap* 1998; **64**:286-8.

108. Kulkarni C, Chalissery P, Jose J, Babba R, Joseph T. Prescribing pattern of antibiotics in abdominal surgery at a teaching hospital: A pilot study. *Indian J Clin Pharmacol Therap* 1994;**10**:4-8.

109. Vaz J, Kulkarni C, David J, Joseph T. Influence of caffeine on pharmacokinetic profile of sodium valproate and carbamazepine in normal human volunteers. *Indian J Exptl Bio* 1998;**36**:112-4.

110. Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas PSSR. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Medica* 1998;**64**:353-6.

111. Roy V, Rewari S. Ambiguous drug pricing: a physician's dilemma. *Indian J Pharmacol* 1998;**30**:404-7.

DRUG DELIVERY SYSTEMS

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1. Introduction

Development of new drug molecule is expensive and time consuming. Improving safety efficacy ratio of "old" drugs has been attempted using different methods such as individualizing drug therapy, dose titration and therapeutic drug monitoring. Delivering drug at controlled rate, slow delivery, targeted delivery are other very attractive methods and have been pursued very vigorously.

This article reviews the work done in our country on drug delivery system. It is interesting to note that considerable work and many publications from USA, Europe are authored by Indian researchers.

2. Transdermal drug delivery system (TDDS)

The literature on transdermal delivery of drugs has been revised[1]. The transdermal drug delivery system has potential advantages of avoiding hepatic first pass metabolism, maintaining constant blood levels for longer period of time, decrease side effects, decrease gastrointestinal effect that occur due to local contact with gastric mucosa and improved compliance.

The release pattern from TDDS is studied by *in vitro* (*ex vivo*) models using artificial membranes or animal or cavdaveric skin. The hairless guinea pig and brattleboro rat appear to be good models for investigating skin/transdermal drug delivery systems, *in vivo*.

Rao and Diwan[2] developed ethylcellulose polyvinyl pyrrolidone (PVP) film containing diltiazem hydrochoride and indomethacin. The influence of initial drug concentration, film composition, and film thickness on the *in vitro* drug release rate as well as drug permeation through rat abdominal skin were studied using paddle over skin method and full thickness rat abdominal skin using a modified Franz diffusion cell. The release rate increased linearly with increasing drug concentration and PVP fraction in the film but was independent of film thickness, followed a diffusion controlled model but a burst effect was observed initially at high concentration. The *in vitro* skin permeation profile showed increased flux values with increased drug and PVP concentration in the film. Authors concluded that films composed of ethylcellulose :PVP:diltiazem (8:2:2) or indomethacin (8:2:3) with suitable adhesive layer and backing membrane could be developed for therapeutic purpose.

The same group studied[3] permeability of cellulose acetate (CA) free films casted from chloroform solution containing different plasticizers *viz.* dibutyl pthalate (DBP), polyethylene glycol (PEG) and propylene glycol (PG). Permeability characteristics of free films were studied using the drugs such as diltiazem hydrochloride (DLT) and indomethacin (Ind). The order of permeability of plasticized films with plasticizers is PEG>PG>DBP. Diffusion of drugs through the free films of CA was extended over a longer period of time at a controlled rate and thus, these can be used as rate controlling membranes for the development of a transdermal drug delivery system.

In an attempt to develop TDDS for pulsatile delivery of testosterone (T) Misra *et al*[4] developed two formulations, one consisting of T and a polymer blend dissolved in isopropanol administered by dispensing the solution on skin to cast a film in situ and another one an adhensive dispersion patch. *In vitro* release from the patch was evaluated using a flow through cell interfaced with a HPLC pump and UV detector. Single dose pharmacokinetics were evaluated in castrated Wistar rats and bonnet monkeys immunized against gonadotropin releasing hormones to deplete endogeneous T. The two formulations resulted in a burst and sustained phase of drug release.

Membrane permeation controlledTDDS for nifedipine using collagen (from calf fetus skin) and chitosan membranes has been developed[5]. Alginate gel was used to increase stability of nifedipine in the system. Transdermal devices were prepared by adhesive sealing technique. *In vitro* release studies were carried out using modified Franz diffusion cells. Drug release was found to depend on the type of membranes used to control the drug delivery.

In another study[6] Chitosan membranes with different permeability to propranolol hydrochloride were obtained by controlled cross-linking with glutaraldehyde to regulate the drug release in the devices. Chitosan gel was used as the drug reservoir. The ability of these devices to deliver the drug while supported on rabbit pinna skin was tested by conducting *in vitro* studies in modified Franz diffusion cells. The drug release profiles showed that the drug delivery is completely controlled by the devices. The rate of drug release was found to be dependent on the type of membrane used.

Krishna *et al* [7] formulated a carboxy methylcellulose sodium based TDD for propranolol and evaluated it for *in vitro* and *in vivo* performance. *In vitro* studies using excised hair free rat skin model in a modified Franz diffusion cell resulted in 66.54% permeation at the end of 24 hr in a zero order permeation profile. Skin irritation studies in rats evaluated for flare and wheal with respect to a formalin control indicated that drug containing patch invoked only a mild response over a 7 day period.

Bhat *et al* [8] prepared betamethasone dipropionate ointment and studied the effect of permeation enhancers such as surfactants, B cyclodextrin, bile salt, iontophoresis, sonophoresis and hyperthermia, using Sigma dialysis membrane and rat skin. Histamine wheal suppression technique was used to assess permeation. It was seen that *in vivo* 0.2% Span 20 and 0.1% sodium lauryl sulphate and 0.45% sodium chloride promoted permeation of drug; sonophoresis and iontophoresis enhanced permeation through rat skin.

Iontophoresis involves transport of ionic (charged) molecule into a tissue by passage of a direct electric current through an electrolyte solution containing ionic molecule to be delivered using an appropriate electrode polarity, and enhancement of transport of high molecular weight peptides and nonelectrolytes due to the indirect effect of electric current *i.e.* coupled flow of water (ion to hydrokinesis). The relative conductivity of skin is proportional to its water content, *stratum cornium is the major barrier for permeation* of ionic compounds. Conventional direct current (dc) iontophoresis inevitably develops a skin polarization potential which reduces the efficacy of iontophoretic transdermal delivery. It may also cause irritation and burns. A prolonged iontophoretic delivery therefore cannot be used.

These limitations can be overcome by using depolarizing or pulsatile current. Nanda *et al* [9] showed that iontophoresis caused a significant increase in transdermal permeation of propranolol hydrochloride *in vitro* through skin. They also studied[10] the effect of pulsed dc wave form on transdermal delivery of propranolol hydrochloride in rats having normal blood pressure and in those made hypertensive with in fusion of noradrenaline. Unlike after passive diffusion, propranolol, delivered *in vivo* into rat by iontophoresis, was detected in blood and it blocked response to isoprenaline and decreased the rise in mean arterial pressure due to noradrenaline.

Transdermal (TD) films of terbutaline hydrochloride was formulated[11] using hydroxypropyl methyl cellulose as monolithic matrix, for evaluation of pharmacokinetic and pharmacodynamic parameters. The skin irritation study revealed no signs of erythema or edema in rabbits. TD formulation was more effective than oral dosage form as evident from the pharmacodynamic studies carried out on guinea pig, using histamine aerosol induced bronchospasm model.

Bioadhesive drug delivery system was prepared[12] using polymer carboxy methyl cellulose, polyvinyl pyrrolidone, sodium alginate, hydroxy propyl cellulose, hyroxymethyl cellulose, 100 cps, K4M, carbopol 93%. The adhesion strength of the polymer was assessed by a shear stress detachment method. The bio-adhesion strength was measured using a modified Martii Marvola method. Hydroxypropylmethyl cellulose K4M and 100 cps tablets exhibited maximum bio-adhension. X-ray studies in rabbits confirmed the bio-adhesion. Tablets had adhesion of more that 7 hours.

Transdermal films to terbutaline sulphate were formulated[13] as monolithic matrices using cellulose polymers like hydroxypropyl methyl cellulose (HPMC), sodium carboxymethyl cellulose (CMC), PEG 400 and propyleneglycol were used as enhancer in various ratios. *In vitro* diffusion studies were carried out across isolated stratum corneum of fresh human cadaver skin using a polycarbonate feeding bottle modified as a diffusion cell. The release of drug from formulation followed a zero order kinetics. The transdermal permeability across human skin was enhanced with the increasing plasticiser concentration. The release rate was greater in case of HPMC films, which may be due to low viscosity and greater hydrophilicity of the polymer than CMC. PEG 400 was found to be better permeation enhancer compared to propylene glycol. The drug permeability was not enhanced when % w/w of plasticizer was doubled from 20 to 40. Combination of plasticizers resulted in significant increase in permeability.

Tatapudy and Madan[14] described preparation of benzolyl peroxide microcapsules. Benzoyl peroxide is used for treatment of acne but it explodes in micronized drug state and is a known skin irritant. Microencapsulation would result in slow release for prolonged period thus avoiding toxic effect.

3. Liposomal and targetted drug delivery system

Liposomes are concentric bilayered structures made of amphipathic phospholipids and depending on the number of bilayer, liposomes are classified as multilamellar (MLV), small unilamellar (SUVs) or large unilamellar (LUVs). They range in size from 0.025µ - 10µ in diameter. The size and morphology of liposomes are regulated by the method of preparation and composition. Liposomes are used for delivery of drugs, vaccines and genes for a variety of disorders.

3.1. Infectious diseases

Bacchawat and coworkers developed liposomal amphotericin and investigated it in animal models of fungal infection and leishmaniasis. Kshirsagar and coworkers[15,16] modified the formulation, developed a "Patient Worthy" sterile pyrogen free liposomal amphotericin preparation and investigated it in patients with systemic fungal infections and leishmaniasis. It was found to be safe producing significantly less adverse effects compared to plain amphotericin in patients with systemic fungal infection, did not produce nephrotoxicity and could be given to patients with renal damage. It was effective in patients resistant to fluconazole and plain amphotericin. Unlike Ambisome (USA) which needs to be used in dose of 3 mg/kg/day this is effective at 1 mg/kg/day dose. The same group studied different dosage regimens of liposomal amphotericin using Aspergillus murine model[17]. It was found that liposomal amphotericin was more effective than equal dose of free amphotericin B given after fungal spore challenge. A large single dose of liposomal amphotericin was more effective, whether given before or after spore challenge, than given as two divided doses.

It was investigated[18] in patients with visceral leishmaniasis and found to be effective in patients who had not responded to antimony, pentamidine and amphotericin. Because of its safety, it can be given at 3 mg/kg/day dose thus reducing total duration of treatment. It was successfully used in a child suffering from visceral leishmaniasis[19]. This is the first liposomal preparation developed outside of USA, which has been used in patients.

In an attempt to improve efficacy and reduce toxicity further, liposomes with grafted ligand have been developed. Pentamidine isethionate and its methoxy derivative were encapsulated in sugar grafted liposomes and tested against experimental leishmaniasis *in vivo* [20]. It was seen that sugar grafted liposomes specially the mannose grafted ones were potent in comparison to normal liposome encapsulated drug or free drug.

3.2. Anticancer drugs

Mukhopadhyay[21] developed conjugate of antineoplastic drug daunomycin (DNM) with maleylated bovine serum albumin. It was taken up with high efficiency by multi drug resistant variant JD100 of the murine - macrophage tumor cell line J774A.1 through the scavenger receptors resulting in cessation of DNA synthesis.

A thermosensitive liposomal taxol formulation (heat mediated targeted drug delivery) in murine melanoma was developed and studied by another group of workers[22]. Cremophor which is used as excipient due to the low aqueous solubility of taxol has toxic side effects. Temperature sensitive liposomes encapsulating taxol were prepared using egg phosphatidylcholine and cholesterol in combination with ethanol. The liposomes have a phase transition temperature of 43°C. A significant reduction in tumor volume was noted in tumour bearing mice treated with a combination of hyperthermia and theromosensitive liposome encapsulated taxol, compared to animals treated with free taxol with or without hyperthermia in B16F 10 murine melanoma transplanted into C57Bl/6 mice.

Sharma *et al* [23] also investigated the use of polyvinylpyrrolidone nanoparticles containing taxol prepared by reverse micro-emulsion method. The size of nanoparticle was found to be 50-60 nm. The antitumor effect of taxol was evaluated in B16F10 murine melanoma transplanted in C57 B 1/6 mice. *In vivo* efficacy of taxol containing nanoparticles as measured by reduction in tumor volume and increased survival time was significantly greater than that of an equivalent concentration of free taxol.

3.3. Lung specific drug delivery

In Chandigarh lung specific liposomes was developed and investigated in animal models of tuberculosis[24].

Liposomes tagged with O.stearlylamylopectin (O-SAP) resulted in increased affinity towards lung tissue of mice. Liposomes containing egg phosphatidylcholine cholesterol dicetylphosphate, O-SAP, monosialo-ganglioside (GMI)/DSPE PEG 2000 were found to be more stable in serum. These liposomes accumulated more in lungs than in reticulo endothelial system of normal and tuberculosis mice. Liposomes were stable *in vivo* and released contents slowly. INH and rifampicin encapsulated in liposomes were less toxic to peritoneal macrophages and also *in vivo* as compared to free drug.

3.4. Targeting to brain

Jain *et al*[25] developed dopamine hydrochloride bearing positively charged small liposomes by sonicating multilamellar vesicles and studied their physical attributes and drug leakage and release pattern. *In vivo* performance was assessed by periodic measurement of chlorpromazine induced catatonia in Sprague Dawley rats and was compared with plain dopamine hydrochloride, dopamine and levodopa carbidopa. The studies showed that dopamine can be effectively delivered into the brain and its degradation in circulation can be prevented by incorporating it into liposomes.

3.5. Transdermal delivery

Bioadhesive liposomes bearing levonorgestrel as controlled drug delivery system has been studied[26]. Mesophasic proliposomal system for levonorgestral was prepared[27]. The vesicles were mostly unilamellar and some were multilamellar. Release was of zero order kinetics. Alcohol as compared to oils had greater effect on transdermal flux.

In vivo studies showed that a significant lag phase was observed before the therapeutic levels were reached indicating the requirement for a loading dose. This proliposomes system was found to be superior to PEG based ointment system.

Liposomal reservoir system bearing local anesthetic benzocaine was developed[28] for controlled and localized delivery via topical route. The liposomal suspension was incorporated into an ointment and gel base. The systems delivered the drug at a controlled rate ever 24 hr compared to plain ointment which had a rapidly decreased release rate. The drug delivery across human cadaver skin was very slow. *In vivo* studies showed a longer duration of action in the case of liposomal formulation.

3.6. Miscellenous

Nabar [29] studied the effect of size and charge of liposome in the bio-distribution of 99mTC-DTPA encapsulated in liposome after intravenous injection in rats. They observed that multilamellar vesicles (MLV) were taken up to a greater extent as compared to SUVs in liver spleen and lungs. Positively charged MLVs than negative or neutral ones, were taken up more in liver, positively charged SUVs were taken up more in kidneys and neutral MLVs were taken up more in lungs than charged ones.

An attempt was made[30] to improve stability of liposome by coupling the drug with the lipid bilayer using a cross linking agent. Soya phosphatidylcholine (SPC) containing liposomes were prepared by calcium induced fusion method. Positively charged stearylamine was introduced in the bilayer. The liposomes were coupled to entrapped ibuprofen by EDAC (1-ethyl 3-(3-dimethyl aminopropyl) carbodiimide HCl) and the coupling was confirmed by UV spectrum. It was observed that EDAC in SPC containing stearylamine liposomes retarded the release of ibuprofen significantly.

In albino rats, the various factors affecting systemic absorption of nasally applied gentamycin sulphate using in situ nasal perfusion technique was studied[31].

Tween 80 which is a surfactant increases permeation by altering membrane structure and permeability. In this study Tween 80 upto 1% W/V concentrations, increased permeability. Betacyclodextrin at 0.25% W/V concentration, another permeability enhancer was found to significantly increase permeability initially but was found to plateau off later on. However both these permeability enhancer were found to decrease stability and potency of gentamycin.

4. Other controlled drug delivery systems

Extended release, slow release and sustained release preparation have been developed by pharmaceutical industry and pharmacy departments and investigated *in vitro* for release pattern and *in vivo* for bio-equivalence.

4.1. Oral

There is a great need in oral delivery of protein and peptide drugs, suitable devices for delivering the therapeutic agent incorporated microspheres selectively in the intestine. Gelatin capsules were coated with various concentrations of sodium alginate and cross-linked with appropriate concentrations of calcium chloride and tested *in vitro* for resistance to gastric and intestinal medium. Gelatin capsules coated with 20% w/v of the polymer, which gave the most promising result *in vitro*, were evaluated in human volunteers for their *in vivo* gastro intestinal tract behaviour. The radiographical studies show that while the un-coated gelatin capsules disintegrated in the stomach within 15 min of ingestion, the alginate coated gelatin capsules remained intact as long as they were retained in the stomach (up to 3 h) and then migrated to the ileocecal region of the intestine and disintegrated[32].

Vanarase and Nagarsenkar[33] prepared pellets of 1mm and 1.65 mm size of prochlorperazine maleate using a modern pelletization technique. The pellets were coated with ethylcellulose and evaluated for *in vitro* release, using USP dissolution apparatus. They noted that release of PCPM can be reduced with increasing amount of ethylcellulose.

Rangaiah *et al* prepared and studied[34] the sustained release tablets of theophylline using Eudragit RLPM, RSPM and HPMC. Bioavailability studies in volunteers showed that HPMC and Eudragit formulation produced sustained plasma concentration of the drug. Another group [35] formulated sustained release capsules of nifedipine containing an initial rapidly available loading dose in the form of solid dispersion and a sustained release part as micro particles coated with polyvinyl acetate (m.wt 45000) film using a modified Wurster coating apparatus. The products provided release of initial therapeutic dose of drug in less than 45 min and sustained release over 11-12 hours. The same group developed[36] a diffusion cell for the determination of drug release from a topical aerosol formulation.

4.2. Parenteral

Kushwaha [37] used a blend of synthetic polymer polyvinyl alcohol and natural macromolecule gum arabica and found that duration and release of drug depends on the amount of drug loaded in the matrix and solubility of the drug in the matrix and the release medium. The advantage of this system is that the release kinetics of the drug from the system can be tailored by adjusting the plasticizer, homopolymer and cross linker composition.

Chitosan microspheres of 45-300 micros were used for controlled delivery of progesterone [38]. *In vitro* and *in vivo* release was tested. It was seen that highly cross linked spheres released only 35% of incorporated steroids in 40 days compared to 70% from lightly cross linked spheres. Determination of *in vivo* bioavailability of the steroid from microsphre formulation by intramuscular injection in rabbits showed that a plasma concentration of 1-2 ng/ml was maintained upto 5 months without a high burst effect. The data suggests that cross linked chitosan microspheres would be an interesting system for long term delivery of steroids.

Cross linked dextran beads were developed as a carrier for development of a single contact vaccine delivery system[39].

There has been extensive research on drug delivery by biodegradable polymeric devices since bioresorbable surgical sutures entered the market two decades ago. Among the different classes of biodegradable polymers, the thermoplastic aliphatic poly (esters) such as poly (lactide) (PLA), poly (glycolide) (PGA), and especially the copolymer of lactide and glycolide referred to as poly (lactide-co-glycolide) (PLGA) have generated tremendous interest because of their excellent bio-compatibility, biodegradability, and mechanical strength[40]. They are easy to formulate into various devices for carrying a variety of drug classes such as vaccines, peptides, proteins and micromolecules. Most importantly, they have been approved by the United States Food and Drug Administration (FDA) for drug delivery.

Dhiman and Khuller[41] found that mice immunized with microparticles of poly (DL-lactide-co-glycolide) (DL-PLG) as delivery vehicles for 71-KDa cell wall associated protein of mycobacterium tuberculosis H37 Ra, exhibited significantly higher T cell stimulation and cytokine release in comparison to 71-KDa emulsified in Freund's incomplete adjuvant (FIA) as well as BCG vaccinated group. Further the protective effect of 71KDa- PLG was compared with 71KDa FIA on the basis of survival rates and viable bacilli load in different organs at 30 days post challenge and median lethal dose(LCD50) of M. tuberculosis H37Rv. 71KDa PLG was more effective when challenge was given 16 week after immunization. Further 71KaDa-PLG immunized group exhibited a significantly higher clearance of bacterial load from the lungs and livers in comparison to the 71KDa FIA immunized group.

Poly (lactide-co-glycolide) (PLG) was used[42] to deliver diclofenac in the form of microspheres and *in situ* gel-forming systems, subcutaneously. The pharmacokinetic and pharmacodynamics studies in the adjuvant - induced arthritic rats showed that micro spheres produced steady therapeutic levels of the drug in the plasma for about 16 days following a single subcutaneous injection. The *in situ* gel-forming provided significantly higher maximum plasma concentration and inhibition of inflammation was maintained for about 10 days.

4.3. Dental product

Somayaji *et al*[43] used an ethylcellulose strip as delivery medium for tetracycline and metronidazole to reduce sub-gingival microorganisms in periodontal pockets. Patients were given supragingival scaling and then divided into 5 groups, depending on the length of time the medication was in place. Sites were marked for tetracycline, metronidazole, and placebo. Sites were wiped and isolated, and baseline microbiology samples were taken for gram staining and culture methods. After treatment, subgingival microbiological samples were taken again. The ethyl cellulose strips were removed and analyzed for any remaining drug. Results showed that tetracycline and metronidazole could both be applied locally to periodontal sites using ethyl cellulose strips and markedly suppress the subgingival bacteria over a period of several days. The tetracycline showed a faster release; however, the metronidazole required a lesser concentration to achieve complete reduction of the subgingival flora.

A saliva activated bio-adhesive drug delivery system was developed[44] for lidocaine hydrochloride and compared its effect with topical gel preparation in dentistry. It was found that DDS adhered to gingival within a minute and produced peak effect in 15 minutes and produced greater depth of anesthesia than the marketed topical gel.

4.4. Colon specific drug delivery

Specific targeting of drugs to the colon is recognized to have several therapeutic advantages. Drugs, which are destroyed by the stomach acid and / or metabolized by pancreatic enzymes, are slightly affected in the colon, and sustained colonic release of drugs can be useful in the treatment of nocturnal asthma, angina and arthritis. Treatment of colonic diseases such as ulcerative colitis, colorectal cancer and Crohn's disease is more effective with direct delivery of drugs to the affected area. Likewise, colonic delivery of vermicides and colonic diagnostic agents require smaller doses.

Prasad *et al*[45] developed a colon specific oral tablet using guar gum as carrier. Drug release studies under conditions mimicking mouth to colon transit have showed that guar gum protects the drug from being released completely in the physiological environment of stomach and small intestine. Guar gum at pH 6.8 is susceptible to colonic bacterial enzyme action, with drug release. Pre-treatment of rats orally with aqueous dispersion of guar gum for 3 days, induced enzyme specifically acting on guar gum. Thereby increasing drug release. The result indicates usefulness of guar gum as a potential carrier for colon specific drug delivery.

A novel colon specific drug delivery system based on a polysaccharide, guar gum was evaluated[46] in healthy human male volunteers, with gamma scintigraphic study using technetium 99m-DTPA as tracer. It was seen that some amount of tracer present on the surface of the tablets was released in stomach and small intestine and the bulk of the tracer present in the tablet mass was delivered to the colon. The colonic arrival time of the tablets was 2-4 hr. On entering the colon, the tablets were found to degrade.

Methacryloyloxy azobenzene and hydrogel was prepared by copolymerizing with hydroxyethyl methacrylate[47]. *In vitro* release studies of the incorporated 5-flurouracil was carried out in simulated gastric and intestinal fluids. *In vitro* release profile in hte presence of azoreductase in the culture of intestinal flora followed a zero order pattern.

5. Conclusion

Pharmaceutical development of drug delivery system is being pursued enthusiastically in many laboratories in India. These are being investigated *in vitro* for release pattern and in some cases *in vivo* in animals for pharmacokinetics but less frequently for efficacy. There is a paucity of data on clinical studies and utility of the DDS in patients. It is necessary that pharmacologists should be involved in the investigation of pharmacokinetics and pharmacodynamics of DDS if the products have reach their meaningful out come - the clinical use.

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REFERENCES

1. Panchagnula R. Transdermal delivery of drugs. *Indian J Pharmacol* 1997;**29**:140-56.
2. Rao PR, Diwan PV. Formulation and *in vitro* evaluation of polymeric films of diltiazem hydrochloride and indomethacin for transdermal administration. *Drug Dev Indian Pharmac* 1998;**24**:327-36.
3. Rao PR, Diwan PV. Permeability studies of cellulose acetate free films for transdermal use: influence of plasticizers. *Pharmaceutica Acta Helvetiae* 1997;**72**:47-51.
4. Misra A, Pal R, Majumdar SS, Talwar GP, Singh O. Biphasic testosterone delivery profile observed with two different transdermal formulations. *Pharm Res* 1997;**14**:1264-8.
5. Thacharodi D, Rao KP. Rate-controlling biopolymer membranes as transdermal delivery systems for nifedipine: development and *in vitro* evaluations. *Biomaterials* 1996; **17**:1307-11.
6. Thacharodi D, Rap KP. Development and *in vitro* evaluation of chitosan- based trandermal drug delivery system for the controlled delivery of propranolol hydrochloride. *Biomaterials* 1995;**16**:145-8.
7. Krishna R, Pandit JK. Carboxymethylcellulose-sodium based transdermal drug delivery system for propranolol. *J Pharm Pharmacol* 1996;**48**:367-70.
8. Bhat M, Shenoy DS, Udupa N, Srinivas CR. Optimization of delivery of betamethasone - dipropionate from skin preparation. *Indian Drugs* 1995;**32**: 211 -4.
9. Nanda A, Khar RK. *Drug Dev Indian Pharm* 1994;**20**: 3033-44.
10. Nanda A, Khar RK. Pulsed mode constant current iontophonetic transdermal delivery of propranalol hydrochloride in acute hypertensive and normotensive rats. *Indian Drugs* 1998;**35**:274-80.
11. Murthy SN, Shobha Rani HS. Comparative pharmacokinetic and pharmacodynamic evaluation of oral vs transdermal delivery of terbutaline sulphate. *Indian Drugs* 1998;**35**:34-6.
12. Rao MY, Vani G, Chary BR. Design and evaluation of mucoadhesive drug delivery systems. *Indian Drugs* 1998; **35**:558-65.
13. Murthy NS, Satheesh M. Enhancer synergism of propylene glycol and PEG -400 in terbutaline sulphate transdermal drug delivery systems. *Indian Drugs* 1997; **34**:224-6.
14. Tatapudy H, Madan PL. Benzoyl peroxide microcapsules I. preparation of core material. *Indian Drugs* 1995;**32**: 239-48.
15. Kshirsagar NA, Gokhale PC, Pandya SK. Liposomes as drug delivery system in leishmaniasis. *J Assoc Physicians India* 1995;**43**:46-8.
16. Kshirasagar NA, Bodhe PV, Kotwani RN. Targeted drug delivery in visceral leishmaniasis. *J Par Dis* 1997;**21**:21-4.
17. Kotwani RN, Gokhale PC, Kshirsagar NA, Pandya SK. Optimizing dosage regimens of liposomal amphotericin B using Aspergillus murine model. *Indian J Pharmacol* 1996;**28**:88-92.
18. Gokhale PC, Kshirsagar NA, Khan MU, Pandya SK, Meisheri YV, *et al.* Successful treatment of resistant visceral leishmaniasis with liposomal amphotericin B. *Trans Roy Soc Trop Med Hyg* 1994;**88**:228.
19. Karande SC, John Boby KF, Kshirsagar NA. Successful treatment of antimony - resistant visceral leishmaniasis with liposomal amphotericin B (L-amp-LRC) in child. *Trop doctor* 1995;**25**:80-1.
20. Banerjee G, Nandi G, Mahato SB, Pakrashi A, Basu MK.

Drug delivery system: targeting of pentamidines to specific sites using sugar grafted liposomes. *J Antimicrob Chemother* 1996;**38**:145-50.

21. Mukhopadhyay A, Mukhopadhyay B, Basu K. Circumvention of multidrug resistance in neoplastic cells through scavenger receptor mediated drug delivery. *FEBS letters* 1995;**276**:95-8.

22. Sharma D, Chelvi TP, Kaur J, Ralhan R. Thermosensitive liposomal taxol formulation: heat-mediated targeted drug delivery in murine melanoma. *Melan Res* 1998;**8**:240-4.

23. Sharma D, ChelviTP, Kaur J, Chakravorty K, DeTK, Maitra A, Ralhan R. Novel taxol formulation : polyvinylpyrrolidone nanoparticle-encapsulated taxol for drug delivery in cancer therapy. *Oncol Res* 1996;**8**:281-6.

24. Deol P, Khuller GK. Lung specific stealth liposomes: stability, biodistribution and toxicity of liposomal antitubercular drugs in mice. *Biochimica Biophysi Acta* 1997;**1334**: 161-72.

25. Jain NK, Rana Ac, Jain SK. Brain drug delivery system bearing dopamine hydrochloride for effective management of parkinsonism. *Drug Dev Ind Pharm* 1998;**24**:671-5.

26. Uppadhyay AK, Dixit VK. Bioadhesive liposomes bearing levonorgestrel as controlled drug delivery system. *Pharmazie* 1998;**53**:421-2.

27. Deo Mr, Sant VP, Parekh SR, Khopade AJ, Banakar UV. Proliposome-based transdermal delivery of levonorgestrel. *J Biomat App* 1997;**12**:77-88.

28. Singh R, Vyas SP. Topical liposomal system for localized and controlled drug delivery. *J Dermatol Sci* 1996;**13**:107-11.

29. Nabar SJ, Nadkarni GD. Effect of size and charge of liposomes on biodistribution of encapsulated ^{99m}Tc - DTPA in rats. *Indian J Pharmacol* 1998;**30**:199-202.

30. Sivakumar PA, Mythily S, Alamelu S, Rao PK. Liposome - ibuprofen conjugate and the release characteristics of ibuprofen. *Indian Drugs* 1994;**31**:568-73.

31. Martin DB, Udupa N. Nasal drug delivery of gentamycin sulphate. *Indian Drugs* 1994;**31**:365-69.

32. Narayani R, Rao KP. Polymer-coated gelatin capsules as oral delivery devices and their gastrointestinal tract behaviour in humans. *J Biomat Sci* Polymer Edition. 1995;**7**: 39-48.

33. Vanarase SY, Nagarsenkar MS. *In-vitro* release studies of prochlorperazine pellets coated with ethylcellulose. *Indian Drugs* 1995;**32**:134-8.

34. Rangaiah KV, Madhusudhan S, Verma PRP. Sustained release of theophylline from HPMC and Eudragit tablet. *Indian Drugs* 1995;**32**:543-7.

35. Asgar A, Sharma SN. Sustained release through coated microparticles of nifedipine. *Indian Drugs* 1996;**33**:30-5.

36. Asgar A, Radha S, Agarwal SP. Fabrication of a diffusion cell for the determination of drug release from topical aerosol formulations. *Indian Drugs* 1997;**34**:715-7.

37. Kushwaha V, Bhowmick A, Behera BK, Ray AR. Sustained release of antimicrobial drugs from polyvinylalcohol and gum arabica blend matrix. *Art Cells Blood Subst Immobilization Biotechnol* 1998;**26**:159-72.

38. Jameela SR, KumaryTV, Lal AV, Jayakrishnan A. Progressive loaded chitosan microspheres: a long acting biodegradable controlled delivery system. *J Cont Rel* 1998;**52**:17-24.

39. Diwan M, Misra A, Khar RK, Talwar GP. Long-term high immune response to diphtheria toxoid in rodents with diphtheria toxoid conjugated to dextran as a single contact point delivery system. *Vaccine* 1997;**15**:1867-71.

40. Jain R, Shah NH, Malick AW, Rhodes CT. Controlled drug delivery by biodegradable poly (ester) devices : different preparative approaches. *Drug Dev Indian pharm* 1998; **24**:703-27.

41. Dhiman N, Khuller GK. Protective efficacy of mycobacterial 71-KDa cell wall associated protein using poly (DL-lactide-co-glycolide) microparticles as carrier vehicles. *FEMS Immunol Med Microbiol* 1998;**21**:19-28.

42. Chandrashekar G, Udupa N. Biodegradable injectable implant systems for long term drug delivery using poly (lactic-co-glycolic) acid copolymers. *J Pharm Pharmacol* 1996; **48**:669-74.

43. Somayaji BV, Jariwala U, Jayachandran P, Vidyalakshmi K, Dudhani RV. Evaluation of antimicrobial efficacy and release pattern of tetracycline and metronidazole using a local delivery system. *J Periodontol* 1998;**69**:409-13.

44. Taware CP, Mazumdar S, Pendharkar M, Adani MH, Devarajan PV. A bioadhesive delivery system as an alternative to infiltration anesthesia. *Oral Sur Oral Med Oral Pathol Oral Radiol Endodontics* 1997;**84**:609-15.

45. PrasadYV, KrishnaiahYS, Satyanarayana S. *In vitro* evaluation of guar gum as a carrier for colon-specific drug delivery. *J Cont Rel* 1998;**51**:281-7.

46. Krishnaiah YS, Satyanarayana S, Rama Prasad YV, Narasimha Rao S. Gamma scintigraphic studies on guar gum matrix tablets for colonic drug delivery in healthy human volunteers. *J Cont Rel* 1998;**55**:245-52.

47. Shantha KL, Ravichandran P, Rao KP. Azo polymeric hydrogels for colon targeted drug delivery. *Biomat* 1995; **16**:1313-8.

DRUG METABOLISM STUDIES IN ANIMAL MODELS

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1. Introduction

In a drug discovery program, accurate pharmacokinetic and metabolic data of the drug must be available all most as early as the results of *in vitro* biological screening. Many of the currently available drugs have one or more metabolites of pharmacological significance. Pharmacokinetically the metabolite may differ in distribution and clearance from the parent drug. An understanding of the kinetics of active metabolite formation is important not only for predicting the therapeutic outcome but also for explaining the toxicity of specific drugs. The advent of commercial liquid chromatography-mass spectrometry instrumentation and the development of high field nuclear magnetic resonance as well as liquid chromatography-nuclear magnetic resonance techniques have further strengthened the ability to study the metabolism of new drugs in early drug discovery stage. The purpose of this review is to highlight the work carried out by various research groups in India from 1994-98 in pharmacokinetics in animal models.

The major laboratories which are engaged in this area include Pharmacokinetics and Metabolism division, Central Drug Research Institute, Lucknow; Dept. of Pharmacology, All India Institute of Medical Sciences New Delhi; Andhra University, Vishakapatnam Dept. of Pharmacology, Post Graduate Institute of Medical Education and Research, Chandigarh; Industrial Toxicology Research Centre, Lucknow; LM College of Pharmacy, Ahmedabad; Regional Research Laboratory, Jammu besides others. For convenience the review will focus on pharmacokinetic in different species, and metabolism studies undertaken separately.

2. Metabolism studies

Significant progress was achieved in this area and the techniques existing in this area were applied to gain a good insight into the mechanisms occurring at the cellular level and probable beneficial and deleterious effects. The application of this field has been listed under separate headings.

3. Toxicity studies

Due to the ethical constraints on performing toxicity studies in humans, relevant safety assessment has been studied in laboratory animals, although extrapolation of risk assessment from animals to human is still challenging. Activities of various drug metabolizing enzymes such as NADPH dependent cytochrome P450 monooxygenase (mixed function oxidases, MFO), glutathione-S-transferase, epoxide hydrolase and several other enzymes (present in liver and several other extrahepatic tissues) have been influenced by factors such as environmental pollutants, industrial solvents, age and sex of the animals.

Basu *et al*[1] studied the effect of lead administration upon coadministration with phenylbutazone in rats. The half life ($t_{1/2}$) of the drug was found to be 33% lower on chronic lead exposure and 46% higher on acute exposure than in unexposed control rats. The study therefore, supported the hypothesis that the inhibition of cytochrome P_{450} is transient and time dependent and that long term exposure might cause induction of liver microsomal enzymes that are responsible for the metabolism of drugs. This study is significant since lead exposed persons require therapy for treatment of other underlying diseases.

Alterations in hepatic and extrahepatic protein content, activities of drug enzymes, lipid peroxidation and hydrogen peroxide formation and levels of microsomal electron transport components were examined in developing chicken during treatment with phenobarbital (PB), benzene, DDT treated groups of 4-8 week old chicken[2]. Activities of hepatic and extrahepatic (kidney, lung, intestine) tissue enzymes were induced during PB treatment in all groups of chicken. However benzene treated groups of chicken recorded decrease in activities of drug enzymes. Magnitude of increase due to PB treatment was much more in liver as compared to kidney, lung and intestine. Hydrogen peroxide and lipid peroxide formation significantly increased in all tissues during treatment with benzene and DDT. Levels of cytochrome P_{450}, cytochrome b_5 and cytochrome reductase, lipid peroxidation and hydrogen peroxide formation increased in all groups of chicken during various treatments. The results suggest that chicken liver contains greater drug enzyme activities and electron transport components during development as compared to other tissues. Benzene and DDT administration resulted into decrease in the activities of drug metabolizing enzymes.

4. Food and drug modulatory effects

Food is known to alter the metabolism of xenobiotics. The effect of food restrictions at different levels on MFOs and the magnitude of their induction by phenobarbital (PB) and 3-methylcholanthrene (3-MC) in adult male and female rats was studied by Ahmed *et al*[3]. In both PB and 3-MC treatment the magnitude of increase in microsomal protein, cytochrome b5, aminopyrine-N-demethylase (APND) activity was less in fasting rats (FR) than in *ad libitum* fed; while cytochrome P_{450} levels and activities of cytochrome reductase and acetanilide hydroxylase (ACOH) was higher in FR animals. NADPH dependent lipid peroxidation and cytosolic glutathione-S-transferase activity were also enhanced due to PB and 3-MC treatment but the magnitude of increase was less in FR animals. The ACOH activity increased to a greater extent than APND activity in FR animals following PB and 3-MC treatment. It was proposed that restricted feed intake resulted in induction of a particular population of cytochrome P_{450} which may be more resistant to catabolic degradation.

Ramana *et al*[4] studied the differential effect of phenobarbitone (PB) on hepatic and renal glutathione-S-transferases (GSTs) in Rhesus monkeys and rodents. The various electrophiles conjugate with glutathione in the presence of GSTs which facilitates their elimination. It was found that PB decreases the activity of hepatic GSTs significantly and that of renal GSTs non-significantly in Rhesus monkeys. These observation are surprising in the context that PB is a well known inducer of GSTs in rodents suggesting that species differences could be a reason for the above findings.

In another interesting study, the effect of 6-MFA (Sixth mycelial acetone fraction from mould fungus *Aspergillus ochraceus*), an interferon inducer, on mixed function oxidase system and N-demethylation in rat hepatic microsomes was evaluated by Dhuley *et al*[5]. Interferon inducing agents including tilorane are known to depress hepatic mixed function oxidase system. It was found that aminopyrine-N-demethylase, which develops at much faster rate, was depressed whereas acetanilide hydroxylase was induced. The implications of the present findings suggest that the drug treatment, specially to patients suffering from viral infection, may induce deleterious effects or toxic manifestations due to decreased metabolism of drugs. These studies show that it is essential to monitor drug levels in blood during interferon treatment.

5. Drug interactions

Coadministration of several drugs to a patient often results in unpredictable therapeutic outcome. Often it is either diminished therapeutic efficacy or increased toxicity of one or more of the administered drugs.

Interaction studies between herbal remedies and modern drug are important since it is common in India for patients to take herbal remedies simultaneously with modern drugs and often without the knowledge of treating physicians. Carbamazepine (CBZ) a narrow therapeutic range drug, is given for a long period to epileptic patients and during the treatment with CBZ the patient may take herbal remedies for other ailments. The influence of co-administration of septilin (commonly used for its antibacterial, antiinflammatory properties) on the pharmacokinetics (pkt) of CBZ was investigated in rabbits by Garg *et al*[6]. It was found that septilin decreased CBZ plasma concentrations during the absorption phase and not in the elimination phase which was however not statistically different.

Vetrichelval *et al*[7] studied the effect of rifampicin on the anti-diabetic activity of fenugreek popularly known as methi. Fenugreek powder became ineffective in both normal and diabetic rats on concurrent administration of rifampicin suggesting that caution may be applied while using rifampicin in diabetic patients.

Trikatu, a combination of *Piper longum, Piper nigrum* and *Zingiber officinalis* (1:1: w/w), is an essential ingredient of numerous formulations of Ayurveda. Its active principle, piperine, has been shown to enhance the bioavailability of drugs like rifampicin and sulphadiazine etc. Piperine has shown to inhibit arylhydrocarbon hydroxylation, ethylmorphine-N-demethylation and 3-hydroxy benzo(a)pyrene glucuronidation in a non specific and noncompetitive manner. Investigation into the possible effect of piperine on the activity of isoniazid was carried out in rats by Kanar *et al*[8]. The study gains significance from the fact that the therapeutic efficacy of isoniazid depends more upon the peak plasma concentration of isoniazid attained rather than the period for which minimum inhibitory concentration is maintained. It was found that piperine reduced the rate and bioavailability of isoniazid. The possible reduction in peak concentration may be due to delay in gastric emptying time. However, this study results needs to be confirmed in humans.

Satyanarayana *et al*[9] studied the influence of pargyline (MAO inhibitor) on pharmacokinetics and hypoglycemic activity of tolbutamide in rabbits. Pargyline treatment (75 mg/kg, *i.p.*) for 3, 7 and 14 days in rats reduced hepatic microsomal ethyl morphine N-demethylase activity and cytochrome P_{450} content in isolated liver microsomes. Pargyline had no influence on the oral absorption and distribution of tolbutamide in rabbits. However, the drug delayed the elimination of tolbutamide. Since tolbutamide is entirely (almost 98%) converted to hydroxy tolbutamide in liver microsomes and part of it is subsequently converted into carboxy tolbutamide by cytosol enzymes, the delay in the elimination might be due to the inhibition of its hepatic metabolism. The prolonged $t_{1/2}$ of *iv* administered antipyrine (a model for assessing the hepatic drug metabolising enzyme activity *in vivo*) in rabbits in the presence of pargyline also confirmed the same. It was found that it increased the $t_{1/2}$ and AUC_{0-4} of tolbutamide resulting in prolonged hypoglycemia. The enzyme inhibitory effect of pargyline seemed to be non specific since it inhibited the hepatic microsomal enzymes in addition to MAO which is a non microsomal enzyme.

Aminopyrine and theophylline are mainly metabolized by MFO subfamily CYP1A. Chandrashekar *et al*[10] studied the interaction studies of these drugs with omeprazole. The results indicated that theophylline, which has a narrow therapeutic range, may not require dose adjustment upon coadministration with omeprazole. This study shows that omeprazole may not alter the activity of MFO subfamily CYP1A.

Dwivedi *et al*[11] studied the effect of picroliv on the pharmacokinetics of rifampicin in rats. It was found that pkt and bioavailability of rifampicin was not altered thus indicating that picroliv may be useful in management or prevention of rifampicin induced hepatotoxicity in patients without affecting its bioavailability.

6.Traditional/Herbal remedies

A good number of preparations/products were tested for their hepatoprotective properties/ mechanisms using rat liver hepatocytes and in rat liver *in vivo*. Differential effect of silymarin (a composite name of 3 isomers namely silybin, silydanin and silychristin) on glucuronidation activity was studied by Chrungoo *et al*[12] using 3-OH-benzopyrene as a probe. The rate of glucuronidation, *in vitro*, of 3-OH-benzopyrene was suppressed. However, when administered 70 mg/kg, *i.p.* to rats for 3 hr it increased hepatic uridine diphosphate glucoronic acid (UDPGA) by 2-fold. The results indicated that silymarin elicits differential effects on the rate of glucuronidation and contents of UDPGA in the isolated rat hepatocytes and in liver.

Recently free radical induced lipid peroxidation has gained much importance because of its involvement in several pathologies. Protection of cell membrane from lipid peroxidatioin becomes a necessity to prevent, cure or delay the aforesaid pathologies. Suja *et al*[13] studied the protective effect of Liv.52 and Liv.100 against *in vitro* peroxidation induced by hydrogen peroxide in rat liver homogenate. It was found that the protective effect of these drugs is attributed to the enhanced supply of reduced glutathione that inhibits the deleterious process of lipid peroxidation.

7. Pharmacokinetic studies

Routine pharmacokinetic studies were performed by a number of research workers on goats and cattle[14-20]. Pharmacokinetic evaluation of trilabendazole and its metabolites were performed in sheep and goats by Sanyal *et al*[21]. It was found that pharmacokinetic parameters were same in both species thus ruling out any species differences.

8. New drug development

During this period a few research groups concentrated on the application of pharmacokinetics and metabolism in new drug development. LM-1554 was developed as a new antihyperlipaemic agent by Goyal *et al*[22]. Pharmacokinetic studies in rats revealed that the drug has a very low bioavailability (4%) which was attributed to its poor absorption. The low bioavailability and low volume of distribution of the compound suggest the gastrointestinal tract as the site of action for the antihyperlipaemic activity. This hypothesis was substantiated by the observation that the compound was active orally and inactive parenterally. This finding helped researchers to conclude that LM-1554 prevents absorption of cholesterol from gastrointestinal tract. Paliwal *et al*[23] studied the excretion and metabolism of a new anthelmintic compound (Compound 81/470) using radiolabelled drug. Tissue distribution and excretion were performed after a single 1 mg/kg, *p.o.* or *i.v.* dose in young male rats. 80% of the radioactivity was accounted for in urine and feces while the rest was in different tissues. The tissues that took up most of the radioactivity were skin and muscle, whereas transport of the drug across the BBB was negligible. The distribution and uptake of the drug in the skin was found to be responsible for its efficacy against skin dwelling parasites. Pharmacokinetics of compound 81/470 and the effect of type of formulation (suspension vs solution) was done in calves by Nagaraja *et al*[24]. The results of the study showed with solution formulation the MRT (mean residence time) and serum levels were increased in the body as compared to those obtained with the drug in dispersible powder, suspended in water. Thus solution formulation is more suitable for the treatment of both intestinal and systemic parasitism in ruminants. Route dependent pharmacokinetic studies were also performed by Nagaraja *et al*[25] on compound 81/470 in rats. The compound was administered orally and intraperitoneally. It followed a one compartment model with linear disposition kinetics. Based on C_{max} and AUC values, bioavailability of 81/470 from solution was significantly higher than from suspension. The suspension showed 42.2% absorption from gut while the solution showed more than 90.2% absorption.

9. Conclusion

Pharmacokinetics and metabolism studies are an important part of the corpus of knowledge required to understand the safety and efficacy of potential new drugs. Comprehensive knowledge of pharmacokinetics of a novel compound is essential as adjunct to good duration of action via the appropriate route of administration. As can be found in this review a good number of research workers are focussing on generating basic data on pharmacokinetics and metabolism in animal models. However the discovery of new drugs and the use of existing techniques in discovering new drugs from our vast traditional knowledge is the need of the hour. Integrated work between various research groups can take our country's research output to new heights.

REFERENCES

1. Basu N, Kulshreshta T. Effect of lead exposure on plasma phenylbutazone levels in rats. *Indian J Physiol Pharmacol* 1994;**3891**:61-2.
2. Hatolkar V S, Pawar SS. Mixed function oxidase system in hepatic and intrahepatic tissues of developing chickens following various treatments. *Indian J Exp Biol* 1995;**33**: 48-50.
3. Ahmed SR, Pawar SS. Effect on the dietary lipids on hepatic MFO system of male rats during food restriction. *Indian J Exp Biol* 1997;**35**:46-50.
4. Ramana KV, Kohli KK. Effect of phenobarbitone on the hepatic and renal glutathione-S-transferases in the Rhesus monkey. *Indian J Pharmacol* 1998;**30**:34-7.
5. Dhuley JN, Naik SR. Effect of 6- MFA, an interferon inducer obtained from *Aspergileus ochraceus* on MFO system in rats. *Indian J Exp Biol* 1996;**34**:1010-15.
6. Garg SK, Islam AS, Kumar N. Effect of Septilin - a herbal preparation on pharmacokinetics of carbamazepine in rabbits. *Indian J Physiol Pharmacol* 1998;**42**:527-33.
7. Vetrichelvan T, Kavimani S, Gupta JK, Narshimham CL. Effect of rifampicin on *Trigonella feoenum* graecum (Fenugreek)- induced hypoglycemia in rats. *Indian J Pharm Sci* 1998;**60**:244-5.

8. Kanar SR, Bhargava VK, Garg SK. Effect of trikatu (piperine) on the pharmacokinetic profile of isoniazid in rabbits. *Indian J Pharmacol* 1998;**30**:254-50.

9. Satyanarayana S, Murali Krishna D, Krishnaiah YSR, Visweswaran D. Interaction of pargyline with tolbutamide in rabbits. *Indian J Physiol Pharmacol* 1995;**39**:71-3.

10. Chandrashekar SM, Chakrabarthi A, Garg SK. Pharmacokinetics of antipyrine and theophylline unaltered by multiple dose omeprazole treatment in rabbits. *Indian J Physiol Pharmacol* 1996;**3**:302-5.

11. Dwivedi AK, Rastogi R, Singh S, Dhawan BN. Effect of picroliv on pharmacokinetics of rifampicin in rats. *Indian J Pharm Sci* 1996;**58**:28-32.

12. Chrungoo VJ, Reen RK, Singh K, Singh J. Effects of silymarin on UDP-glucuronic acid and glucuronidation activity in the rat isolated hepatocytes and liver in relation to D-galactosamine toxicity. *Indian J Exp Biol* 1997;**35**:256-63.

13. Suja V, Sharmila SL, Shyamala Devi CS. Protective effect of Liv.52 and Liv.100, Ayurvedic formulation on lipid peroxidation in rat liver homogenate. *Indian J Exp Biol* 1997; **35**:50-3.

14. Roy BK, Yadava KP, Banerjee NC. Effect of pyrogen induced fever on the biokinetics of cefazolin in goats. *Indian J Pharmacol* 1994;**26**:156-8.

15. Lahar C, Gupta PK. Pharmacokinetics of parbendazole in goats. *Indian J Pharmacol* 1994;**26**:235-6.

16. Juliet S, Barman A, Mandal TK, Chakraborty AK. Disposition kinetics of sulphamethoxypyridarine during endotoxin induced fever and its modification by sodium salicylate in goats. *Indian J Pharmacol* 1995;**27**:222-6.

17. Roy BK, Pandey SN, Sinha KP, Singh KK. Pharmacokinetics of pefloxacin in goats. *Indian J Pharmacol* 1997; **27**:435-7.

18. Bhavaskar SK, Malik J K. Pharmacokinetics of metronidazole in calves. *British Vet Journal* 1994;**150**:389-93.

19. Patel HM, Bhavaskar SK, Thaker AM, Malik JK. Pharmacokinetics of metronidazole in sheep following *i.v.* and oral administration. *Indian Vet Jour* 1994;**71**:771-4.

20. Parikh V, Shivprakash, Patel D, Gandhi TP, Patel RB, Santani DD. Effect of aspirin on single and multiple dose pharmacokinetics of ciprofloxacin in rabbits. *Indian J Pharmacol* 1996;**28**:25-9.

21. Sanyal PK. Pharmacokinetics study of tridabendazole in sheep and goat. *Indian J Pharmacol* 1994;**26**:200-3.

22. Chaman Lal J Shishoo, Gandhi TR, Rathod IS, Jam KS, Goyal RK. Mechanism of the antihyperlipaemic activity and pharmacokinetics of 2-chloromethyl-5,6,7,8,-Tetrahydro-(b)thieno(2,3-d)pyrimidine-4(3h)one. *Arzeim Forsch/Drugs Res* 1997;**47**:1125-9.

23. Paliwal JK, Ramesh D, Gupta RC. Synthesis and disposition of 14C-labelled 81/470, A new anthelmintic agent in rats. *Indian J Clin Pharm Res* 1997;**107**:23-30.

24. Nagaraja NV, Singh SK, Jain GK, Singh S, Gupta RC. Preliminary observations on the pharmacokinetics of CDRI compound 81/470 in calves. *Vet Res Commun* 1998;**22**:67-72.

25. Nagaraja NV, Singh SK, Jain GK, Singh S, Gupta RC. Pharmacokinetics of Methyl N-[5[[4-(2-Pyridinyl)-1-piperazinyl]carbonyl]-1H-benzimadazol-2-yl]-carbamate after intraperitoneal and oral administration in rats. *Pharmaceutical Sci* 1995;**1**:321-4.

REVIEW OF ENDOCRINE PHARMACOLOGY

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1. Introduction

Research publications in the field of endocrinology from Indian scientists, who conducted the work in an Indian laboratory, during 1994-1998, have been analysed. In endocrine pharmacology, the main areas of Indian scientist's interest were diabetes mellitus and reproductive system. However, very few research laboratories continue to work in this field. In this review we have mentioned the name of the institutes, only if they have published three or more papers in the same field. Since we focused more on the pharmacological research, some of the clinical reports are not included in the review.

2. Diabetes mellitus

In diabetes mellitus, major contributions were in the field of hypoglycemic action of various plant products and drug interaction of hypoglycemic agents. Alloxan or streptozotocin (STZ) induced diabetic animal models were used in most of the studies.

2.1. Herbal drugs

The herbal formulation D-400, which is the combination of *Eugenia jambulana, Pterocarpus marsupium, Ficus glomerulata and Ocimum sanctum* with other herbs, showed a favourable response to alloxan induced hyperglycemia and renal damage in rabbits[1]. The increased glucose level, suppressed glycogen level and *in vitro* decreased ^{14}C-glucose uptake by liver slices in diabetic rats were brought to within normal levels by D-400[2]. The glucose tolerance of D-400 was also studied in STZ-induced diabetic rats[3].

Significant antidiabetic and antihyperlipaemic effect was reported with neem seed (*Azadirachta indica*) kernal powder in alloxan diabetic rats[4]. Possible mechanism of antihyperglycemic effect of neem leaf extract was studied in normal and STZ induced diabetic rabbits. The reduction in peripheral utilization of glucose and glycogenolytic effect due to epinephrine action was blocked by the extract almost completely in diabetic rabbits and to certain extent in normal ones[5].

A significantly high level of metabolic enzyme, malate dehydrogenase was observed in STZ induced diabetic rats compared to control. Insulin as well as the leaf extract of *Aegle marmelose* treatment brought about a reversal of the Km values of these enzyme to near normal. Moreover, *A. marmelose* was found to be as effective as insulin in restoration of blood glucose and body weight to normal levels[6].

Chronic administration of crude aqueous extracts of *Momordica charantia* Linn. and *Swertia chirayita* Buch. showed hypoglycemic effect in STZ treated rats and mice. *S. chirayita* was found to be more effective on chronic treatment[7].

The aqueous extract of *Zizyphus jujuba* showed hypoglycemic activity in alloxan induced diabetic rats[8]. Aqueous extract of *Syzigium cumini* (Jamun) showed hypoglycemic and antioxidant property in alloxan diabetic rats[9].

Trigonella foenum graecum (fenugreek) produced a significant dose related fall in blood glucose level both in the normal as well as diabetic rats[10]. It also significantly reduced various serum lipids in normal rats and decreased the increased levels of lipids and HDL cholesterol in the diabetic rats[11].

Aqueous and ethanol extracts of *Caesalpinia bonducella* seeds produced hypoglycemic effect in normal rats and antihyperglycemic activity in STZ-diabetic rats. The drug also showed a hypolipidemic activity in diabetic rats[12].

Methanol extract of the aerial parts of *Artemisia pallens* wall significantly reduced the blood glucose level of glucose-fed hyperglycemic and alloxan induced diabetic rats. The water extract was inactive[13].

The hypoglycemic effect of *Inula racemosa* was not found to be due to the enhanced secretion/synthesis of insulin; the action may be at the peripheral level by potentiating the insulin sensitivity[14].

Antidiabetic treatment with powdered fruit of *Capparis decidua* lowered alloxan induced lipid peroxidation and altered erythrocyte superoxide dismutase and catalase enzymes to reduce oxidative stress in alloxan diabetic rats[15]. Tarakeswara rasa, an Ayurvedic drug significantly reduced the blood glucose level, cholesterol, triglycerides and phospholipid contents in alloxan diabetic rats[16].

Oral administration of *Prunus amygdalus* seeds (almond seed) produced significant hypoglycemic effect in albino rabbits[17]. The extract of root of *Gynandropsis gynandra* produced hypoglycemic activity in a dose dependent manner when administered to albino rats[18].

Augusti and co-workers from Amala cancer research center, Trichur studied the hypoglycemic effect of *Ficus bengalensis* Linn (Indian banyan tree). Leucodelphinidin and leucopelargonin derivative isolated from the bark of Indian banyan tree demonstrated hypoglycemic action in both normal and alloxan diabetic rats[19] and dogs[20] respectively.

Three phenolic constituents from *Pterocarpus marsupium* (Vijayasar) showed significant antihyperglycemic activity in STZ-induced diabetic rats[21]. The study of the insulin mimetic effect of (-) epicatechin, a benzopyran extracted from bark of Vijayasar, showed that insulin and (-) epicatechin act by different mechanisms of action while eliciting their protective effects on human red cell osmotic fragility[22]. Clinically a flexible dose open trial was undertaken in four centers in India to evaluate the efficacy of Vijayasar in the treatment of newly diagnosed or untreated NIDDM. The results suggested that, Vijayasar is useful in the treatment of diabetes mellitus[23].

Leaf extract of *Ocimum sanctum* and *Ocimum album* (holy basil) on fasting and post prandial blood glucose and serum cholesterol levels in humans was studied through randomized placebo controlled cross over single blind trial. A significant decrease in fasting and post prandial blood glucose levels observed during treatment with holy basil leaves when compared to treatment with placebo[24].

The hypoglycemic activity of more plants including phyllanthus amarus, *Salacia oblonga wall, Camellia sinensis, Lantana camara and Trasina,* an Ayurvedic formulation have been discussed by Dr. Dahanukar.

2.2. Synthetic drugs

2.2.1. Hypoglycemic action

Studies by Satyanarayana and co-workers have suggested the involvement of nitric oxide (NO) in tolbutamide activity and the possibility of using L-arginine as a supplement to antidiabetic drugs in blood glucose control[25]. Paragyline[26] but not ranitidine[27] significantly increased the elimination half-life and AUC of tolbutamide, which resulted in prolonged hypoglycemia[26]. However ranitidine enhanced the hypoglycemic activity of glybenclamide[27].

Cimetidine potentiated and prolonged the hypoglycemic activity of glibenclamide when both were administered concomitantly in normal and alloxanized rabbits[28]. Effect of doxycycline on quinine induced hypoglycemia was studied in albino rats. Doxycycline treatment antagonised the hypoglycemic effect of quinine[29].

Amitriptyline produced significant hyperglycemia within 4 hours and attained a peak at 24 hours in 18 hour fasted albino rabbits. The drug also produced glucose intolerance during early hours[30]. Acute and chronic use of terfenadine and astemizole produced hypoglycemia in patients with allergic rhinitis[31].

The hypoglycemic effects of chlorpropamide, metformin and tolbutamide complexes with Co (II) and Zn (II) were found to be more effective than the parent drugs[32].

2.2.2. Opiate receptor and glucose

Number of studies were published with the antagonistic action of glucose on opiate receptors. Male Wistar rats with STZ induced diabetes delayed and reduced antinociceptive effect of morphine compared to control group[33]. Significant analgesic activity was produced both in diabetic and control rats after immobilization stress. The vocalization threshold was however not altered in diabetic rats, probably due to the antagonistic action of glucose on opiate receptor at the level of brain stem[34]. Met-enkephalin raised blood glucose level and decreased muscle and liver glycogen contents in albino rats. Naloxone produced significant reversal of the effects of met-enkephalin[35].

Acute administration of felodipine produced significant analgesia in both control and diabetic rats. Both acute and chronic administration of felodipine significantly potentiated the morphine analgesia in diabetic rats[36].

2.2.3. Other studies

The inhibitory effect of loperamide on gastrointestinal transit was assessed in acute and chronic hyperglycemic states. Loperamide produced significant inhibition of gastrointestinal transit in chronic and acute hyperglycemic rats as compared to their respective saline treated control[37]. Inhibition of digestive and absorptive function was observed in a combined state of vitamin A deficiency and diabetes mellitus in albino rats[38].

The onset and progression of cataract in diabetic rat was delayed by aspirin and sodium salicylate; but aspirin effect was more pronounced than that of sodium salicylate. Acetylation may be playing a major role in aspirin's anticataract effect *via* inhibition of glycation[39].

Digoxin level in plasma was found to be increased in diabetic patients. Experiments revealed that the cause of the increase in digoxin levels may be a tendency towards mild hypothyroidism associated with diabetes mellitus[40].

Insulin induced hypoglycemic convulsive response was studied in male and female mice. The response was similar in male and female mice. However compared to female mice, male mice appeared to be more susceptible to lower ambient temperature (17-25°C) and the latent phase was comparatively lower[41].

The (^{125}I) insulin binding and receptor kinase activity were assessed in rat brain in the presence of a β agonist, isoproterenol. While insulin binding remain unaltered, isoproterenol treatment significantly enhanced receptor kinase activity in control and hyperglycemic conditions[42]. Increased activity of hexokinase and decrease in phosphoglucoisomerase[43] and cytosolic dehydrogenase[44] activities were observed in erythrocytes of insulin dependent diabetic rats.

Studies on diabetic rats revealed that increased oxidative stress and accompanying decrease in natural protective antioxidants *viz.* glutathione and ascorbic acid may be related to the causation of diabetes mellitus[45,46]. Vitamin C supplementation was effective to some extent in maintaining the levels of plasma and liver ascorbic acid and liver glutathione46.

In order to assess the β-cell function, a reliable method was standardized for performing sensitive intravenous glucose test. A normal curve for glucose disposal was also constructed in monkeys (*Macaca raidata radiata*)[47].

Alloxan diabetes reduced the glucose transport across the cell membrane Na^+-K^+ ATPase in Swiss mice[48].

2.2.4. Diabetes and hypertension

Goyal and his co-workers, L.M.College of Pharmacy, Ahmedabad have made significant contribution in the field of diabetes with hypertension. The effect of different antihyper-tensives in STZ induced diabetic rats have been evaluated in detail, and the findings of the animals studies further established by controlled open clinical trials. This topic is extensively covered in the chapter on cardiovascular pharmacology.

3. Reproductive endocrinology

3.1. Male reproduction

3.1.1. Herbal drugs

Researchers from Karnataka extensively studied the effect of *Azadirachta indica* on reproductive system of albino rats. Study of the histological and biochemical changes in the testis after treatment with neem leaves and its pattern of recovery, revealed its possible reversible antiandrogenic property[49]. Neem leaves produced histological and biochemical changes in the caput and cauda epididymis[50]. Oral administration of the dry leaf powder resulted in decrease in the weight of seminal vesicles and ventral prostate, reduction in epithelial height and nuclear diameter and the secretory materials in the lumen[51].

The antiandrogenic or antispermatogenic effect of the ethanolic extract of *Striga orobanchioides* was studied in male rats. The drug decreased the weight of the testes, epididymis, seminal vesicles and the ventral prostate. It also produced degenerative changes in Leydig cells, their nucleii, the seminiferous tubules and a significant decrease in the number of spermatocytes and spermatids[52].

3.1.2. Synthetic drugs

Levels of both serum testosterone and renal β-glucuronidase activity were significantly decreased in cisplatin-treated rats[53]. The decreased activity of renal β-glucuronidase activity was prevented by supplementation with testosterone[54].

Administration of exogenous gonadotrophins to the rats treated with pethidine for 30 days significantly reversed the pethidine suppressed gonadal activities. Gonadotrophin treatment increased the weight of testis, diameter of testis and somniferous tubules and stimulated the spermatogenetic activity, which was suppressed by pethidine[55].

Haloperidol, a known antidopaminergic agent, increased serum prolactin level in male rats. At testicular level, it produced the suppression of hypophyseal gonadotrophin. This confirmed the importance of dopaminergic control over prolactin release for normal function of the gonad[56]. The atrophic changes in the principal cells of orchidectomised rats were significantly reversed when prolactin was administered. Prolactin may have a rejuvenating effect on epididymal principal cells in androgen deficient states[57].

Cyclophosphamide decreased testis and cauda epididymal weight, sperm count, motile and viable spermatozoa and increased percentage of abnormal spermatozoa. The levels of lipids and total cholesterol were not affected[58]. Administration of graded doses of nicotine for 5 days to adult mice significantly reduced the weight of testis, number of spermatocytes, and number of spermatids. It also reduced the weight of sex organs, which are dependent on androgens produced by the testis[59].

Fluoxetine treatment to male patients with sexual dysfunction produced significant improvement in the symptoms of premature ejaculation. The drug did not produce significant anticholinergic side effects[60].

3.1.3. Castration

Castration of male albino rats resulted in significant reduction of pain threshold. The reduction in pain threshold was brought back to normal by substitution with testosterone therapy[61]. Testosterone reversed the decreased concentration of ascorbic acid-2-sulphate in tissues of castrated rats[62].

Decreased testosterone level by castration produced a decrease in neurotransmitters like GABA, NA, 5HT and dopamine, compared to the intact animals. However estrogen administration also produced a decrease in neurotransmitters. It suggested that testosterone and estrogen produce opposite effect on the level of the neurotransmitters in rat CNS[63].

The heart rate recorded from partially restrained estrous female rat, before and after the snout contact by the normal rat, showed a significant rise. There was no such rise in response to the snout contact by neonatally gonadectomised male rat of the same age. Testosterone propionate administration to gonadectomised male rat also could not increase the heart rate of female rats. This may be due to the perturbation in the structure of sexually dimorphic medial preoptic area (MPOA) in the male rat as a result of neonatal gonadectomy [64].

3.1.4. Opioid peptides

Systemic administration of opioid peptides, methionine-enkephalin and β-endorphin, chronically lowered gonadotrophin level in plasma and had an inhibitory effect on the testicular enzymes. When rats were treated with opioid peptide antagonist naloxone and N-acetyl β-endorphin antiserum, it induced the opposite effect[65]. Intratesticular administration also suggested that opioid peptides as well as N-acetyl β-endorphin have some role in regulating reproductive functions at gonadal level[66].

3.1.5. Toxic Chemicals

Tetramethyl thiuram disulfide (Thiram), a commonly used fungicide administration to male rats caused marginal increase in the relative weight of testis and epididymis and decrease in the weight of seminal vesicle and prostate. Marked degenerative changes were observed in seminiferous tubules together with alteration in testicular enzyme profile[67].

Chronic exposure of male albino rats to formaldehyde decreased the sperm motility, viability count and DNA content in prostate and testis. The long term occupational exposure to formaldehyde may lead to male sterility[68]. Single administration of malathion significantly reduced plasma concentration of both

LH and testosterone in adult male rats. Prior administration of human chorionic gonadotrophins (hcG) for 2 days protected the malathion induced hormonal changes[69]. Phosphamidone, a neurotoxic insecticide was tested for male reproductive toxicity with special reference to epididymis. Phosphamidone affected the principle cells indirectly through its toxic effect on the Leydig cells[70].

3.1.6. Human studies

Increase in concentration of potash alum decreased the time for death/complete immobility of sperm, collected from healthy volunteers[71]. Alcohol induced perturbation on the functional integrity of the testis, thyroid and adrenal were observed in male alcohol addicts. However, 20 days period of total alcohol abstinence and rehabilitative program failed to reverse alcohol-induced hypoandrogeni-zation and altered thyroid status, but only restored certain biochemical events associated with the excretion of steroid metabolites[72].

3.2. Prostate gland

The prostate gland plays an important role in male reproduction. It secretes enzymes, lipids, amines and metal ions essential for the normal function of spermatozoa[73].

Kumar *et al.* from All India Institute of Medical Science, New Delhi, has published many research papers on receptor transcripts in prostate gland. The receptors of the steroids viz. androgen, estrogen and progesterone were quantitated in the cytosolic and benign hypertrophied (BPH) and carcinomatous (CaP) prostate samples. The study strongly suggested the estrogen-dependent positive regulation of cytosolic androgen receptor[74].

3.2.1. Detection of mRNA

Androgen receptor mRNA (AR mRNA) was detected in normal, BPH and CaP samples using a cDNA probe[74]. AR mRNA could also be detected in the urogenital sinus of human foetuses at 12 and 16 weeks of gestation. This confirmed the androgen mediated differentiation and development of the prostate[75].

RNA of androgen receptor, epidermal growth receptor (EGFR mRNA) and basic fibroblast growth factor (FGFR2 mRNA) could be detected in the autopsy specimen of prostate by slot blotting. However, the integrity of RNA was lost in the sample. The study established the feasibility of detecting or extracting RNA from human prostate postmortem[76].

3.2.2. Analysis of mRNA in BPH and CaP

Studies in human prostate tissue samples from normal, CaP and BPH subjects showed that, AR mRNA was positive in the majority of cases in all 3 groups. All the patients who had received endocrine treatment were also positive for AR mRNA. The level of AR mRNA was significantly higher in BPH and treated CaP cases as compared to normal cases. Among CaP cases the level of AR mRNA was significantly higher in the treated group as compared to the untreated group[77].

In a similar study, EGFR mRNA was positive in majority of CaP cases but only in 50% of normal and BPH cases. The level of EGFR mRNA was significantly higher in the untreated CaP group as compared to the normal group, while the difference in the normal and BPH group was not statistically significant[78].

3.2.3. Animal studies

Experiments were carried out to evaluate the effect of androgen deprivation on the level of androgen receptors transcriptors in the ventral prostate of adult male Wistar rats. Androgenic deprivation was achieved by treatment with estradiol benzoate, flutamide and with a GnRH analogue. When analysed, AR mRNA showed significant rise following treatment with estradiol benzoate and flutamide and a transient rise with GnRH analogue. The result indicated that androgenic deprivation up-regulates the level of receptor transcript[79].

3.2.4. Prazocin

The efficacy of prazocin treatment in BPH was clinically evaluated in 26 patients. The result suggested that the drug is effective in the treatment of BPH with minimal adverse effects[80].

3.3. Female reproduction

Chronic administration of phenobarbitone inhibited the ovarian compensatory hypertrophy significantly and increased the cholesterol and lipid levels in the ovary. Administration of FSH alone or in combination with LH restored the ovarian compensatory hypertrophy and decreased cholesterol and lipid levels significantly. However LH alone was not much effective[81].

Light microscopic studies displayed increased atresia, cessation of oogenesis and ovulation, degeneration of follicular cells of zona pellucida and corona radiata in zinc deficient BALB mice. Zinc deficiency may lead to changes in the secretion of hormones in mice and may slow growth or arrest ovulation or atresia of the growing follicle in the ovary[82].

The changes in the reproductive system of immature female rats were studied after intraperitoneal administration of urinary protein of pregnant women. There was a significant increase in the uterine weight. However, there was no change in the weight of ovary, adrenal, kidney, liver and spleen of the rats[83].

Treatment of female rats with 17-β-estradiol increased the ovarian catalase activity[84]. *In vitro* addition of 17-β-estradiol resulted in synthesis and release of a number of proteins in human fallopian tube. Addition of progesterone leeds to inhibition of protein synthesis and the combination of both drugs negated the effect[85].

Polymorphic granulosa cells of ovary of house rat showed a wide spectrum of variation in atropine and testosterone propionate induced atresia[86]. The direct involvement of nicotine on reproduction was studied in albino mice. The study revealed the antagonistic action of nicotine to gonadotrophin[87].

Chronic ethinyl estradiol treatment of albino rats did not alter the pD_2 values of nor-adrenaline in isolated aortic strip; however, it produced significant increase in pD_2 values of nor-adrenaline in portal vein preparation. The treatment did not alter the aortic relaxation[88].

Fertility and parturition indices were reduced in rats treated with monocrotophos, an organophosphate pesticide. However, gestational index or average litter size were not affected. Viability and lactation indices were highly reduced in rats of high dose groups[89]. Edifenphos, an organophosphorus fungicide produced a dose dependent effect on rats in the number of healthy follicles and atretic follicles in different stages[90].

3.4. Contraceptives

The validity and usefulness of oral contraceptives began in 1953 with the fundamental work of Puncus and Chang. The female sex hormones, estrogen and progestins have played an outstanding role after that[91]. Despite progress in new and improved methods of contraception over past few decades, efforts are still being made to reduce adverse effects and to enhance patient compliance.

All contraceptive methods are not equally effective. Many factors including, mechanism of action, ease of use, side effects and availability determine the actual effectiveness[92].

3.4.1. Herbal Drugs

Many indigenous plants were used by Ayurvedic physicians in India from time immemorial for prevention of conception. Very little scientific data is available regarding the nature of the active components and their mechanism of action.

Nigella sativa, a herb widely cultivated in North India, was tested for its post-coital contraceptive efficacy in rats. Hexane extract of the seeds prevented pregnancy in rats. Significant antifertility activity was also observed in its column fraction and subfractions[93]. Benzene extract of the anthers of *Mahela champaka* and ethanolic extract of the seeds of *Centratherum anthelminticum* also showed significant post-coital anti-implantation activity in rats[94].

Trans anethole, the major constituent of anise oil from *Illicium anisatum*, was shown to be capable of producing antifertility activity in rats. The drug showed a dose-dependent activity and a 100% anti-implantation activity at 80mg/kg, *p.o.* A significant estrogenic activity was also produced but it did not possess anti-estrogenic, progestational, anti-progestational, androgenic or anti-androgenic activities[95].

One of the potent antifertility agents, ethanolic extract of *Bupelerum marginatum* (silpi) has been studied to assess its estrogenic activity in immature rats. The extract significantly increased the uterine weight and caused opening and cornification of vagina in immature rats. Histological features of uterus seemed to be highly stimulated[96]. Hexane extract of *Ferula jaeschkena* commonly known as “Heeng” is well known for its antifertility activity. Administration of the extract to adult cyclic guinea pigs showed duration dependent luteolytic changes in the corpora lutea. Ovarian wet weight, proteins and glycogen contents were decreased while the activity of acid phosphatase in the ovary was increased[97].

Aqueous extract of the leaves of *Angle Marmelos* was studied for its abortifacient activity in albino rats. The drug did not show any anti-implantation activity, but showed a dose-dependent abortificient activity. Abortificient activity was increased with advancement of pregnancy[98].

3.4.2. Male contraceptives

Extracts of the flower of *Hibiscus Rosasinensis* administration to albino mice resulted in the decrease of spermatogenic elements of testis and epididymal sperm count. The drug also showed androgenic activity in immature mice[99].

3.4.3. Non Herbal Drugs

Effect of methoxy progesterone acetate (MPA) alone and MPA with testosterone enanthalate were investigated on rat vas deferens and fertility for 60 days. Altered histopathology and contractile pattern of vas deferens resulted in reduced fertility. The drug showed androgen antagonistic effects but the effects were found to be reversible[100].

Experiments were carried out to identify the effective period of luteal phase (luteal phase window) when a single administration of mifepristone would induce antinidatory activity without disturbing menstrual cyclicity and ovulatory pattern in the rhesus monkey. The duration of the effective period of early luteal phase contraception with mifepristone was found to be wider (days 2-5 after ovulation). The study revealed that there was no change in cycle length, day of ovulation and duration of menses compared with mifepristone treatment and post treatment cycles[101].

The long acting nature of norethisterone enanthate (NET-En), a well known intramuscular contraceptive drug, was evaluated in female rats, hamster[102], rabbits, monkeys and in women[103] after oral administration. The study showed that NET-En was absorbed within a day after oral administration. A dose dependent reduction in fertility was seen in rats when they mated after 5 days of drug administration. However, the effect was less pronounced and not significant when the rats mated 10 days after the drug treatment. In hamsters, a significant but less pronounced result was noted. Compared to intramuscular administration, the relative oral bioavailability of NET-En ranged from 10-51% in rabbits, monkeys and women. This suggested that NET-En could be useful as a long acting oral pill.

The anti-implantation activity of immunomodulatory bone marrow cytokine (BIM) was studied in adult rats. The result showed that up regulation of the immune system by BIM at the time of implantation evoked a cellular response in uterus that prevented implantation. The anti-implantation effect of BIM was reversible[104].

Danazol, a synthetic steroid derivative of 17-β-ethinyl testosterone, induced biochemical transformation in the ovarian tissue of rats possibly through the hypothalamic pituitary ovarian axis[105].

The possibility that the risk of atherosclerosis complication increases with oral contraceptive use was examined by studying the effect of oral pill containing estrogen and progestin on the metabolism of lipids in female rats fed with atherogenic diet. The increase in cholesterol level in plasma and aorta, increase in LDL+VLDL cholesterol and considerable decrease in HDL cholesterol was observed in the treated animals[106].

The effect of contraceptive use of depot-medroxyprogesterone acetate (DMPA) on levels of serum immunoglobulins, total protein and albumin in Indian women were studied. While the serum levels of IgA, IgM, total protein and albumin were unaffected, the IgG levels were increased in the first and third month after DMPA injection[107].

3.4.4. Centchroman

Centchroman, a biphenyl chroman derivative with weak estrogenic and potent anti-estrogenic activity is the only available non-steroidal oral contraceptive[108].

Scientists of Central Drug Research Institute, Lucknow, extensively studied the pharmacokinetics of centchroman. A precise and sensitive HPLC assay was developed and validated for determination of centchroman and its 7-demethylated metabolite in human serum and milk[109].

Milk excretion of centchroman was studied in nursing mothers. The breast milk and serum centchroman concentration showed a significant correlation indicating that the amount of centchroman excreted into the breast milk was dependent on serum concentration. There was significant increase in centchroman concentration in milk after multiple dosing compared to the single dosing. The level of centchroman passing into breast milk and subsequent exposure of the infants is unlikely to be of any physiological consequences[110].

Pharmacokinetics of single oral dose of centchroman[111] and comparative bioavailability study of two commercial centchroman tablets[108] were conducted in healthy female volunteers. Dose dependent first order kinetics was observed with the drug. Both commercial tablets were comparable without significant variation.

Two injectable formulations of centchroman were compared for *in vitro* release profile, stability and *in vivo* antifertility efficacy in female albino rats. The two formulations were poly (Lactide-co-glycoside) (PGLA)-in-Triacetin and niosome prepared by lipid layer hydration method. The formulation showed controlled drug release and enhanced stability *in vitro* and *in vivo*. PGLA-in-Triacetin showed better antifertility activity[112].

Direct effect of anti-estrogens like centchroman and tamoxifen on motility and penetration ability of human spermatozoa was studied *in vitro*. The motility was invariably reduced after incubation with the drug. When 17-β-estradiol combined with the drugs, inhibition of motility was seen in a dose-related manner. When the anti-androgens reduced the distance travelled by spermatozoa to 30%, the combination of 17-β-estradiol with the anti-androgens reduced it up to 50%[113].

4. Thyroid gland

The effect of *Ocimum sanctum* leaf extract on the changes in the concentration of serum T_3, T_4 and serum cholesterol were investigated in male mouse. While the serum T_4 concentration significantly reduced after drug administration, no marked changes were observed in serum T_3 level, T_3/T_4 ratio and in serum cholesterol level. The study suggested the antithyroid effect of *Ocimum sanctum leaf*[114].

After 10-12 months of carbimazole therapy for Grave's disease in 21 patients, 43% of patients were able to induce long term remission. Around 53% of patients relapsed with in one year after stopping the therapy. An abnormal T_4 suppression test was used as a reliable parameter for predicting relapse[115].

Effect of thyroxin was studied on histamine induced bronchospasm in guinea pigs. Chronic treatment with the drug significantly protected against experimental bronchospasm. Thyroxin also potentiated the salbutamol-evoked broncho-dialatation[116]. The hedonic response to sweetness was tested in 12 thyroxin treated rats using method of single-bottle brief-exposure to sweet solution. Consumption of the sweet solution was significantly more following administration of thyroxin than during the control period[117].

Serum glycoprotein levels were increased in propylthiouracil induced hypothyroid rats and it was depleted in L-thyroxin induced hyperthyroid rats[118].

Scorpion envenomation reduced the time of circulating T_4 and T_3 levels, an autonomic storm releasing massive amount of catecholamine, angiotensin II and suppressed insulin secretion in dogs and rabbits[119]. Serum T_4 and TSH level with respect to blood levels of organochlorine pesticides and occupation were studied among 103 rural population. About 24.3% of subjects showed depleted T_4 levels in association with significantly lower level of organochlorine pesticide residues in blood[120]. Cyanide exposure in workers dealing with cyanide compounds resulted in a decrease in serum T_4 and T_3 concentration and increase in TSH concentration compared to control subjects[121].

Cigarette and bidi smoking significantly decreased serum T_4 level in smokers compared to the nonsmoking subjects in two different studies[122,123]. However, T_3 and TSH levels were not comparable between the two studies. While a nonsignificant increase in T_3 level and significant decrease in TSH level reported with one study[122], a decrease in T_3 level and normal TSH levels with smokers reported in another study[123].

Parathyroidectomy interfered in the pathogenesis of necrosis and associated lesions in the liver of CCl_4 treated rats. Unilateral parathyroidectomy afforded better protection than bilateral parathyroidectomy[124].

5. Adrenal gland

Role of cortisone and regulated carbohydrate energy in mammary gland growth and energy regulated biochemical parameters has been evaluated in the adrenalectomised pre-pubertal female rats. The results suggested that glucocorticoid is an essential growth factor during pre-pubertal age. The hormone appeared to operate via tissue metabolism and stimulating energy intake through appetite stimulation[125].

Effect of adrenalectomy and treatment with hydrocortisone after adrenalectomy on testes was studied in rats. Degenerative changes such as decreased seminiferous tubule diameter, Leydig cell nuclear diameter, spermatogenic arrest, edematous fluid in the interstitium and lumen of seminiferous tubules and increased levels of zinc, copper and enzymes in adrenalectomised rats suggested a possible role of adrenal cortex and its hormones in the changes. Hydrocortisone administration as a single dose acted as hyperstate of hydrocortisone for a short time but once hydrocortisone was metabolised, the animals returned to adrenalectomised state and the degeneration persisted[126]. Administration of high dose of glucocorticoid produced degeneration of epididymis in adult male rats. The study also revealed the involvement of pituitary-testis axis in hydrocortisone induced epididymal degeneration and functional inhibition[127].

Studies in mated rats proved that adrenocorticoids are not obligatory for conceptus development but were important for efficient action of ovarian steroids in the establishment and maintenance of pregnancy[128]. The effect of corticosteroids on imipramine analgesia was evaluated in sham adrenalecomised and adrenalectomised rats using tail flick method. Adrenalectomised rats displayed greater sensitivity to imipramine induced analgesia. Administration of adrenocorticosteroids attenuated the analgesic effect of imipramine[129].

Effect of corticosterone, cortisol, norepinephrine and epinephrine were studied both *in vivo* and *in vitro* on the rate of oxygen consumption of tissues of subtropical Indian frogs. Corticosterone, epinephrine and norepinephrine are directly involved in the regulation of energy metabolism of the frogs[130].

Patients with pulmonary sarcoidosis have peripheral blood lymphopenia and lymphocytic alveolitis. They have increased levels of complement and immunoglobulin both in the peripheral blood and bronchoalveolar lavage fluid. Effect of daily intake of prednisolone was studied in 29 patients with pulmonary sarcoidosis. There was a significant improvement in all the abnormalities with prednisolone treatment[131].

Plasma level of corticosterone was induced by exposure to both acute and chronic noise stresses in albino rats. Treatment of rats with ethanol extract of *Ocimum sanctum* prevented the change in the corticosterone level[132]. Indigenous drugs like *Aloe vera, Angle marmelos, Moringa oleifera* and *Tridax procumber* hastened normal wound healing and reversed the healing suppressant effect of dexamethasone in male Wistar rats[133].

6. Melatonin

Melatonin potentiated the hypnosis induced by phenobarbitone and decreased both total and ambulatory activity. It produced hypothermia and possessed significant analgesic activity. While the antinociceptive effect of melatonin was sensitive to reversal by naloxone, other CNS depressant profile resembled the effects of benzodiazepines[134].

In male albino mice melatonin showed modulatory role in haemopoiesis and its rhythms. The stimulatory effect of melatonin on WBC supported its purported immunopotentiating action[135].

Daily treatment with melatonin for 8 weeks to albino rats proved the influence of melatonin on the level of ocular fluid, structure/function of retina and Harderian gland[136].

Subcutaneous administration of melatonin to 5 and 10 day old rat pups showed an advancement of vaginal opening in 5 day old pups but delayed it in 10 day old pups. In the 5-day-old groups, the ovaries and thymus showed an increase in weights, but the 10-day group recorded a fall. Follicular and thymic corticomedullary ratios also displayed corresponding increase and decrease[137].

7. Conclusion

Though this review is not complete in all the aspects, this gives a clear picture about the mode of research work carried out in India in the field of endocrine pharmacology during 1994-1998. Only few pharmacology departments are concentrating on endocrinology, compared to other departments. Moreover, half the way stopping of the research is also a problem with our research laboratories. For example, number of preliminary studies being carried out on various activities of different plant extracts. However, very few researchers continue their work with the same plant to bring out appreciable products (like morphine, atropine or digoxin) which have clinical significance. A constant effort is required in the same field to produce research work with international quality. However, it is appreciable that, some of our researchers could produce quality works with novel and innovative ideas. We hope in the coming years, more contribution from the Indian researchers will rich this field with quality products.

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REFERENCES

1. Dubey GP, Dixit SP, Alok S. Alloxan-induced diabetes in rabbits and effect of a herbal formulation D-400. *Indian J Pharmacol* 1994;**26**:225-6.

2. Dhawan D, Bandhu HK, Sing B, Ajaib S, Nagpl JP. Effect of D-400 (a herbal formulation) on the regulation of glucose metabolism in diabetic rats. *Indian J Pharmacol* 1996;**28**:224-6.

3. Mitra SK, Gopumadhavan S, MuralidharTS, Anturlikar SD, Sujatha MB. Effect of D-400, a herbomineral preparation on lipid profile, glycated haemoglobin and glucose tolerance in streptozotocin induced diabetes in rats. *Indian J Exp Biol* 1995;**33**:798-800.

4. Bopanna KN, Kannan J, Sushma G, Balaraman R, Rathod SP. Antidiabetic and antihyperlipaemic effects of neem seed kernel powder on alloxan diabetic rabbits. *Indian J Pharmacol* 1997;**29**:162-7.

5. Chttopadhyay RR. Possible mechanism of antihyperglycemic effect of *Azadirachta indica* leaf extract. Part IV. *Gen Pharmacol* 1996;**27**:431-4.

6. Seema PV, Sudha B, Pius SP, Asha A, Reghu KG, Paulose CS. Kinetic studies of purified malate dehydrogenase in liver streptozotocin-diabetic rats and the effect of leaf extract of *Aegle marmelose* (L.) Correa ex Roxb. *Indian J Exp Biol* 1996;**34**:600-2.

7. Banerjee S, Sengupta A, Banerjee J, Adhikary P, Chatterjee A. Studies on hypoglycemic effect of indigenous herbs. *Indian J Pharmacol* 1994;**26**:229-30.

8. Ignacimuthu S, Amalraj T. Effect of leaf extract of *Zizyphus jujuba* on diabetic rats. *Indian J Phrmacol* 1998;**30**:107-8.

9. Prince PS, Menon VP, Pari L. Hypoglycemic activity of *Syzigium cumini* seeds: effect on lipid peroxidation in alloxan diabetic rats. *J Ethnopharmacol* 1998;**61**:1-7.

10. Khosla P, Gupta DD, Nagpal RK. Effect of *Trigonella foenum graecum* (Fenugreek) on blood glucose in normal and diabetic rats. *Indian J Physiol Pharmacol* 1995;**39**:173-4.

11. Khosla P, Gupta DD, Nagpal RK. Effect of *Trigonella foenum graecum* (Fenugreek) on serum lipids in normal and diabetic rats. *Indian J Pharmacol* 1995;**27**:89-93.

12. Sharma SR, Dwivedi SK, Swarup D. Hypoglycemic, antihyperglycemic and hypolipidemic activities of *Caesalpinia bonducella* seeds in rats. *J Ethnopharmacol* 1997;**58**:39-44.

13. Subramoniam A, Pushpangadan P, Rajasekharan S, Evans DA, Latha PG, Valsaraj R. Effects of *Artemisia pallens* Wall. on blood glucose levels in normal and alloxan-induced diabetic rats. *J Ethnopharmacol* 1996;**50**:13-7.

14. TripathiYB, Padmaja C. Assessment of endocrine response of *Inula racemosa* in relation to glucose homeostasis in rats. *Indian J Exp Biol* 1995;**33**:686-9.

15. Poonam Y, Sarkar S, Bhatnagar D. Lipid peroxidation and antioxidant enzymes in erythrocytes and tissues in aged diabetic rats. *Indian J Exp Biol* 1997;**35**:389-92.

16. Vasanthakumari V, Shyamala DCS. Evaluation of antidiabetic effect of an Ayurvedic drug, 'tarakeswara rasa' in rats. *Indian J Exp Biol* 1997;**35**:909-11.

17. Sunita T, Singh M. Hypoglycemic effect of *Prunus amygdalus* seeds in albino rabbits. *Indian J Exp Biol* 1997;**35**:295-6.

18. Rao RP, Babu RM, Rao KRV, Murali GV, Babu M. Studies on antipyretic, analgesic and hypoglycemic activities of root of *Gynandropsis gynandra* Linn. *Indian Drugs* 1997;**34**:690-3.

19. Geetha BS, Biju CM, Augusti KT. Hypoglycemic effects of leucodelphinidin derivative isolated from *Ficus bengalensis* (Linn.). *Indian J Physiol Pharmacol* 1994;**38**:220-2.

20. Augusti KT, Reji SD, Sheeja C, Sheela CG, Sudhakaran NCR. Effect of leucopelargonin derivative from *Ficus bengalensis* Linn. on diabetic dogs. *Indian J Med Res* 1994;**99**:82-6.

21. Manickam M, Ramanathan M, Jahromi MA, *et al.* Antihyperglycemic activity of phenolics from *Pterocarpus marsupium*. *J Nat Prod* 1997;**60**:609-10.

22. Rizvi SI, Zaid MA, Suhail M. Insulin-mimetic effect of (-) epicatechin on osmotic fragility of human erythrocytes. *Indian J Exp Biol* 1995;**35**:791-2.

23. ICMR, Collaborating Centers, New Delhi. Flexible dose open trial of Vijayasar in cases of newly-diagnosed noninsulin-dependent diabetes mellitus. *Indian J Med Res* 1998;**108**:24-9.

24. Agarwal P, Ravi V, Sing RB. Randomized placebo-controlled, single blind trial of holy basil leaves in patients with noninsulin-dependent diabetes mellitus. *Int J Clin Pharmacol Ther* 1996;**34**:406-9.

25. Satyanarayana S, Krishnaiah YSR, Kondala BR. Involvement of nitric oxide (NO) in hypoglycemic activity of tolbutamide. *Indian J Physiol Pharmacol* 1998;**42**:551-4.

26. Satyanarayana S, Murali KD, KrishnaiahYSR, Visweswaran D. Interaction of paragyline with tolbutamide in rabbits. *Indian J Physiol Pharmacol* 1995;**39**:71-3.

27. Krishnaiah YSR, Satyanarayana S, Visweswaram D. Influence of ranitidin on the hypoglycemic activity of glibenclamide. *Indian J Physiol Pharmacol* 1994;**38**:316-8.

28. Patil BM, Lakshman M. Drug interaction of glibenclamide and cimetidine. *Indian J Hospital Pharm* 1994;18-22.

29. Imoba ST. Study on modification of hypoglycemic effect of quinine by doxycycline in albino rats. *Indian J Physiol Pharmacol* 1994;**38**:309-10.

30. Bhavyesh G, Mahesh KS, Ashok K, Bhuwaneshwar PJ. Effect of amitriptyline on blood glucose level in rabbits. *Indian J Physiol Pharmacol* 1995;**39**:279-82.

31. Chakravarti A, Lal A, Vishwakarma SK. Hypoglycemic effect of terfenadine in patients of allergic rhinitis. *Indian J Physiol Pharmacol* 1996;**40**:191-2.

32. Sandeep RN, Nirankar NM, Pratibha P, Ashok K, Pitre KS. Antidiabetic activity of some oral hypoglycemic agents with metal ions on animal model. *Indian J Exp Biol* 1996;**34**:81-2.

33. Arati SM, Krishna C, Tarun KM, Chakrabarty AS. Nociception, antinociceptive potency of morphine in streptozotocin induced diabetic rats. *Indian J Physiol Pharmacol* 1997;**41**:416-20.

34. Arati SM, Krishna C, Tarun KM, Chakrabarty AS. Immobilisation stress induced analgesia in diabetic rats. *Indian J Physiol Pharmacol* 1997;**41**:275-9.

35. Gulati R, Agarval DK, Ali R, Srivastava U, Hussain MM. Effect of met-enkephalin on blood glucose level. *Indian J Physiol Pharmacol* 1998;**42**:559-62.

36. Rani NMA, Chandran U, Andrade C, Venkataraman BV. Felodipine potentiates morphine analgesia in control and streptozotocin diabetic female rats. *Indian J Exp Biol* 1996;**34**:663-6.

37. Patil BM, Taher A. Effect of hyperglycemia on loperamide induced inhibition of gastrointestinal transit in rats. *Indian J Pharmacol* 1995;**27**:269-70.

38. Sinha M, Ghosh AK. Influence of dietary vitamin A deficiency on rat digestive and absorption functions during diabetes mellitus. *Indian J Med Res* 1994;**100**:196-200.

39. Geetha VS, Molly T, Victoria AJ, *et al.* Effect of aspirin and sodium salicylate on cataract development in diabetic rats. *Indian J Exp Biol* 1998;**36**:651-7.

40. Joshi SD, Santani DD, Sheth JJ, Mehta HC, Dave KC, Goyal RK. Investigation into the possible mechanisms involved in altered digoxin levels in diabetic patients. *Indian J Physiol Pharmacol* 1996;**40**:65-9.

41. Garg SK, Ayub MA, Sabir M. Acute refractoriness to insulin-induced hypoglycemic convulsions in mice. *Indian J Pharmacol* 1997;**29**:437-8.

42. Ansari MA, Gopa G, Najma ZB. β-Adrenergic modulation of rat brain insulin receptor activity in normal and hyperglycemic conditions. *Indian J Exp Biol* 1994;**32**:92-4.

43. Dinesh KG, Farooq A, Suhail M. Activation of mild insulin dependent diabetes mellitus in rat erythrocytes. *Indian J Exp Biol* 1997;**35**:532-4.

44. Dinesh KG, Farooq A, Suhail M. Effect of alloxan induced mild insulin dependent diabetes mellitus on erythrocyte cytosolic dehydrogenases. *Indian J Exp Biol* 1996;**34**:262-3.

45. Madhu CG, Ojha S, Bansal DD. Antioxidant status of streptozotocin diabetic rats. *Indian J Exp Biol* 1996;**34**:264-6.

46. Madhu CG, Kiran PS, Devi DB. Effect of vitamin C supplementation on oxidative stress in experimental diabetes. *Indian J Exp Biol* 1997;**35**:264-6.

47. Sathya K, Balamurugan AN, Savithiri S, Soosaiah G. Intravenous glucose tolerance test in *Macaca radiata radiata*. *Indian J Physiol Pharmacol* 1997;**41**:176-8.

48. Mishra G, Routray R, Das SR, Behera HN. Alloxan diabetes in Swiss mice: activity of Na^+-K^+-ATPase and succinic dehydrogenase. *Indian J Physiol Pharmacol* 1995;**39**:271-4.

49. Anjali RJ, Nazeer AR, Pathan KM, Manivannan B. Effect of *Azadirachta indica* leaves on testis and its recovery in albino rats. *Indian J Exp Biol* 1996;**34**:1091-4.

50. Kasturi M, Manivannan B, Nazeer AR, Parveen DS, Pathan KM. Changes in epididymal structure and function of albino rat treated with *Azadirachta indica* leaves. *Indian J Exp Biol* 1995;**33**:725-9.

51. Kasturi M, Nazeer AR, Pathan KM, Parveen DS, Manivannan B. Effects of *Azadirachta indica* leaves on the seminal vesicles and ventral prostate in albino rats. *Indian J Physiol Pharmacol* 1997;**41**:234-40.

52. Hiremath SP, Badami S, Swamy HK, Patil SB, Londonkar RL. Antiandrogenic effect of *Striga orobanchioides*. *J Ethnopharmacol* 1997;**56**:55-60.

53. Malarvizhi D, Mathur PP. Renal. β-glucuronidase activity is a bioassay of serum testosterone levels in rats. *Indian J Exp Biol* 1996;**34**:582-3.

54. Malarvizhi D, Mathur PP. Testosterone propionate prevents cisplastin induced inhibition of renal β-glucuronidase activity in rats. *Indian J Exp Biol* 1996;**34**:483-5.

55. Patil SR, Sonar A, Londonkar R, Patil SR, Patil SB. Efficacy of exogenous gonadotropins on the maintenance of spermatogenesis in pethidine treated albino rats. Indian J Physiol Pharmacol 1998;42:509-14.

56. Mukherjee M, Ghosh A. Influence of haloperidol on testicular functions in rats. *Indian J Exp Biol* 1997;**35**:1014-5.

57. Rajeev G, Meenakshi G, Neeti S, Pereira BMJ. Rejuvenation of epididymal principal cells by prolactin under androgen deficiency. *Indian J Exp Biol* 1994;**32**:854-9.

58. Rajinder D, Sangha GK, Bilaspuri GS. Cyclophosphamide-induced structural and biochemical changes in testis and epididymis of rats. *Indian J Exp Biol* 1997;**35**:771-5.

59. Reddy S, Londonkar R, Ravindra, Reddy S, Patil BS. Testicular changes due to graded dose of nicotine in albino mice. *Indian J Physiol Pharmacol* 1998;**42**:276-80.

60. Raju GRA, Rani PU, Naidu MUR, Ramesh T, Schobha JC. Evaluation of fluoxetine in premature ejaculation. *Indian J Pharmacol* 1997;**29**:204-5.

61. Padnekar JR, Mulgaonker VK. Role of testosterone on pain threshold in rats. *Indian J Physiol Pharmacol* 1995;**39**:423-4.

62. Sreelatha KKT, Menon PV, Leelamma S. Long term administration of testosterone on ascorbic acid metabolism in castrated rats. *Indian J Exp Biol* 1994;**32**:458-60.

63. Das A, Chaudhuri SK. Effects of sex steroids on the concentrations of some brain neurotransmitters in male and female rats: some new observations. *Indian J Physiol Pharmacol* 1995;**39**:223-30.

64. Karth KNB, Ramakrishna T. Heart rate in female rat in response to snout contacts by normal, gonadectomised and testosterone-pretreated gondectomised male. *Indian J Physiol Pharmacol* 1996;**40**:335-9.

65. Lakshmi NA, Vijayan E. Effects of chronic systemic administration of opioid peptides, naloxone and N-acetyl β-endorphin antiserum on gonadotropins and testicular functions in the rat. *Indian J Exp Biol* 1998;**36**:361-6.

66. Lakshmi NA, Vijayan E. Studies on the effect of intratesticular administration of oipoid peptides, naloxone or N-acetyl β-endorphin antiserum on some testicular parameters in rats. *Indian J Physiol Pharmacol* 1998;**42**:107-12.

67. Mishra VK, Srivastava MK, Raizada RB. Testicular toxicity in rat to repeated oral administration of tetramethylthiuram disulfide (Thiram). *Indian J Exp Biol* 1998;**36**:390-4.

68. Pradip KM, Kumar VL. Inhibitory effects of formaldehyde on the reproductive system of male rats. *Indian J Physiol Pharmacol* 1995;**39**:80-2.

69. Prakash N, Venkatesh U. Human chronic gonadotrophin (hcG) protects malathion induced plasma leuteinizing hormone and testosterone changes in rats. *Indian J Pharmacol* 1996;**28**:257-60.

70. Akbarsha MA, Sivasamy P. Male reproductive toxicity of phosphamidon: Histopathological changes in epididymis. *Indian J Exp Biol* 1998;**36**:34-8.

71. Singh HP, Singh CK, Singh RR. Effect of potash alum (aluminum potassium sulphate) on human semen and sperm. *Indian J Physiol Pharmacol* 1998;**42**:311-4.

72. Sudha S, Balasubramanian, Arunakaran J, Govindarajulu P. Preliminary study of androgen, thyroid and adrenal status in alcoholic men during deaddiction. *Indian J Med Res* 1995;**101**:268-71.

73. Kumar VL, Majumdar PK. Prostate gland: Structure, Functions and Regulation. *Int Urol Nephrol* 1995;**27**:231-43.

74. Kumar VL, Majumdar PK, Wadhwa SN. Steroid receptors and transcript for androgen receptor in benign hypertrophy and carcinoma of human prostate. *Proceedings of the international cancer congress. New Delhi, October,* 1994. pp 2167-71.

75. Majumder PK, Kumar VL. Androgen receptor mRNA detection in the human foetal prostate. *Int Urol Nephrol* 1997;**29**:633-5.

76. Kumar VL, Majumder PK, Murty OP, Kumar V. Detection of receptor transcripts for androgen epidermal growth factor and basic fibroblast growth factor in human prostate post-mortem. *Int Urol Nephrol* 1998;**30**:301-4.

77. Majumder PK, Wadhwa SN, Kumar VL. Androgen receptor transcript level in benign hypertrophy and carcinoma of the human prostate. *Urol Int* 1997;**59**:231-5.

78. Kumar VL, Majumder PK, Gujral S, Kumar V. Comparative analysis of epidermal growth factor receptor mRNA levels in normal, benign hyperplastic and carcinomatous prostate. *Cancer Letters* 1998;**134**:177-80.

79. Kumar VL, Majumder PK, Kumar V. Androgen deprivation causes up-regulation of androgen receptor transcript in the rat prostate. *Mol Cell Biochem* 1997;**171**:133-8.

80. Basalingappa S, Xavier IJ, Madappa KM. Clinical evaluation of prozosin in symptomatic benign prostatic hyperplasia - an Indian experience. *Indian J Pharmacol* 1997;**29**:420-5.

81. Aduri PR, Patil SB. Ovarian sensitivity to exogenous gonadotrophins in phenobarbital treated unilaterally ovariectomized albino rats. *Indian J Physiol Pharmacol* 1994;**38**:108-12.

82. Kaswan S, Bedwal RS. Light and electron microscopic changes in the ovary of zinc deficient BALB/c mice. *Indian J Exp Biol* 1995;**33**:469-79.

83. Latha PI, Narayana K, Honnegowda. The effect of urinary protein from pregnant women on reproductive system of female rats. *Indian J Pharmacol* 1995;**27**:250-2.

84. Singh D, Pandey RS. Changes in catalase activity and hydrogen peroxide level in rat ovary during estrous cycle and induction of catalase in rat ovary by estradiol-17β. *Indian J Exp Biol* 1996;**36**:421-3.

85. Shah AS, Rahgavan VP, Nandedkar TD, *et al.* Effect of estrogen and progesterone on newly synthesised and released human fallopian tube specific proteins. *Indian J Exp Biol* 1998;**36**:564-8.

86. Sharma RK, Guraya S. Atropine and testosterone propionate induced atretic changes in granulosa of house rat (Rattus rattus) ovary. *Indian J Exp Biol* 1997;**35**:1166-9.

87. Patil SR, Ravindra, Patil SR, Londonkar R, Patil SB. Nicotine induced ovarian and uterine changes in albino mice. *Indian J Physiol Pharmacol* 1998;**42**:503-8.

88. Bhatt JD, Gulati OD. Effects of chronic treatment of rat with ethinyl estradiol on the sensitivity of isolated vascular preparations to nor-adrenaline. *Indian J Pharmacol* 1996;**28**:113-5.

89. Adilaxmamma K, Janardhan A, Reddy KS. Monocrotophos: reproductive toxicity in rats. *Indian J Pharmacol* 1994; **26**:126-9.

90. Math RJ, Jadaramkunti UC, Kaliwal BB. Effect of edifenphos on follicular dynamics in albino rats. *Indian J Exp Biol* 1998;**36**:39-42.

91. Arthur TH. Allen and Doisy's 'an ovarian hormone'. *JAMA* 1983;**250**:2684-8.

92. Elizabeth C, McGuffey. Contraceptive options for the 1990s. *J Am Pharm Assoc* 1996;**37**:149-53.

93. Keshri G, Singh MM, Lakshmi V, Kamboj VP. Post-coital contraceptive efficacy of the seeds of *Nigella sativa* in rats. *Indian J Physiol Pharmacol* 1995;**39**:59-62.

94. Sharma S, Mehta BK, Gupta DN. Screening of post-coital antiimplantation activity of *Machela champaka* (anthers) and *Centratherum anthelminticum* (seeds). *Indian Drugs* 1994;**31**:280-1.

95. Dhar SK. Anti-fertility activity and hormonal profile of trans-anethole in rats. *Indian J Physiol Pharmacol* 1995;**39**:63-7.

96. Jonathan S, Dehadrai S, Prakash AO. Estrogenic activity in the ethanolic extract of *Bupleurum marginatum*. *Indian J Pharmacol* 1995;**27**:258-61.

97. Pathak S, Jonathan S, Prakash AO. Timely administration of extract of *Ferula jaeschkena* causes luteolysis in the ovary of cyclic guineapig. *Indian J Physiol Pharmacol* 1995;**39**:395-9.

98. Gangadhar R, Lalithakumari K. Abortifacient activity of the aqueous extract of the leaves of *Aegle marmelos* (Bel) in albino rats. *Indian Drugs* 1995;**32**:129-31.

99. Reddy MC, Murthy RKD, Saraswati BP. Antispermatogenic and androgenic activities of various extracts of *Hibiscus rosa sinesis* in albino mice. *Indian J Exp Biol* 1997;**35**:1170-4.

100. Rao MV, Roy GK, Prasannalata SN. Effect of methoxy progesterone acetate and testosterone enanthalate on vas deferens of rats. *Indian J Exp Biol* 1998;**36**:157-61.

101. Ghosh D, Pradip DE, Senguptha J. Leuteal phase contraception with mifepristone (RU 486) in the Rhesus monkey. *Indian J Physiol Pharmacol* 1994;**38**:17-22.

102. Ravinder P, Nair MK, Sivakumar B. Biological efficacy and plasma norethisterone levels of orally administered norethisterone enanthate in rat and hamster. *Indian J Physiol Pharmacol* 1998;**42**:485-90.

103. Ravinder P, Shatrugna V, Nair KM, Sivakumar B. Pharmacokinetics of orally administered norethisterone enanthate in rabbits, monkey and women. *Contraception* 1997;**55**:373-9.

104. Ghoshal D, Bhattacharyya P, Pakrashi A. Anti-implantation effect of a bone-marrow cytokine-BIM. *Indian J Physiol Pharmacol* 1998;**42**:352-8.

105. Sangha GK, Chopra N. Dianazol-induced biochemical changes in the rat ovary. *Indian J Physiol Pharmacol* 1998; **42**:252-8.

106. Sissan GMA, Leelamma S. Role of oral contraceptive in atherosclerosis. *Indian J Exp Biol* 1994;**32**:307-10.

107. Lali P, Chandra L, Gupta RP. Serum immunoglobulin levels during contraceptive use of depotmedroxyprogesterone acetate in Indian women: a preliminary study. *Contraception* 1996;**53**:363-5.

108. Lal J, Nityanand S, Asthana OP, Gupta RC. Comparative bioavailability of two commercial centchroman tablets in healthy female subjects. *Indian J Pharmacol* 1996;**28**:32-4.

109. Lal J, Paliwal JK, Grover PK, Gupta RC. Simultaneous liquid chromatographic determination of centchroman and its 7-demethylated metabolite in serum and milk. *J Chromatogr B Biomed Appl* 1994;**658**:193-7.

110. Gupta RC, Paliwal JK, Nityanand S, Asthana OP, Lal J.

Centchroman: a new non-steroidal oral contraceptive in human milk. *Contraception* 1995;**52**:301-5.

111. Lal J, Asthana OP, Nityanand S, Gupta RC. Pharmacokinetics of centchroman in healthy female subjects after oral administration. *Contraception* 1995;**52**:297-300.

112. Shenoy DB, Udupa N, Singh UV, Kumari N. In-vitro and in-vivo evaluation of novel injectable contraceptive preparations of centchroman. *Indian J Pharmacol* 1997;**29**:233-7.

113. Allag IS, Rangari K. In-vitro effect of estrogen-antagonist on motility and penetration ability of human spermatozoa. *Indian J Exp Biol* 1997;**35**:822-4.

114. Panda S, Kar A. *Ocimum sanctum* leaf extract in the regulation of thyroid function of male mouse. *Pharmacol Res* 1998;**38**:107-10.

115. Goswami R, Bal CS, Gupta N, Kochupillai N. Remission with carbimazole therapy & assessment of T_4 suppression test as an index of relapse in patients with Graves' disease in India. *Indian J Med Res* 1996;**103**:272-7.

116. Das DN, Bagchi D, Mandal S, *et al.* Effect of thyroxin on experimental bronchospasm in guineapigs. *Indian J Physiol Pharmacol* 1995;**39**:290-2.

117. Sircar SS, Bhatia S. Effect of thyroxin on taste hedonics in rat. *Indian J Physiol Pharmacol* 1994;**38**:217-9.

118. Uniyal BP, Singh LR, Nagpal KK. Effects of hypothyroidism and hyperthyroidism of serum glycoproteins in experimental rats. *Indian J Exp Biol* 1998;**36**:148-51.

119. Murthy RKK, Zare AM. Effect of Indian red scorpion (*Mesobuthus tamulus concanesis, Pocock*) venom on thyroxin and triidothyronin in experimental acute myocarditis and its reversal by specific antivenom. *Indian J Exp Biol* 1998;**36**:16-21.

120. Srivastava AK, Gupta BN, Bihari V, *et al.* Organochlorine pesticide exposure and thyroid function: a study in human subjects. *J Environ Pathol Toxicol Oncol* 1995;**14**:107-10.

121. Banerjee KK, Bishayee A, Marimuthu P. Evaluation of cyanide exposure and its effect on thyroid function of workers in a cable industry. *J Occup Environ Med* 1997;**39**:258-60.

122. Banerjee KK, Marimuthu P. Effect of cigarette smoking on thyroid hormone homeostasis. *Indian J Med Res* 1994;**99**:74-6.

123. Behra D, Dash RJ, Suman A. Alterations in circulating thyroid hormones in Indian smokers (bidi and cigarette). *J Assoc Physicians India* 1995;**43**:96-7.

124. Rasgoti S. Effect of carbon tetrachloride on rat liver, following parathyroidectomy: Histopathological observations. *Indian J Exp Biol* 1997;**34**:443-7.

125. Singh A, Mishra S, Rao MK, Paul PK. Regulatory role of cortisone and dietary carbohydrate energy in prepubertal rat mammary gland growth and development. *Indian J Exp Biol* 1997;**35**:27-32.

126. Nair N, Bedwal RS, Mathur. Effect of adrenalectomy + hydrocortisone treatment on histopathological, biochemical and zinc and copper profiles in rat testes. *Indian J Exp Biol* 1995;**33**:655-63.

127. Neena N, Bedwal RS, Mathur RS. Zinc, copper and hydrolytic enzymes in epididymis of hydrocortisone treated rat. *Indian J Exp Biol* 1998;**36**:22-33.

128. Arora R, Mishra S, Paul PK. Role of adrenal in establishment and development of conceptus in unilaterally ovariectomized mated rats. *Indian J Exp Biol* 1994;**32**:382-6.

129. Mandhane SN, Chopde CT, Muthul AV. The effect of adrenal steroids on imipramine analgesia. *Indian J Pharmacol* 1994;**26**:218-21.

130. Gupta BBP, Mahanta A. Involvement of adrenal hormones in tissue respiration of sub-tropical hibernating and non-hibernating species of frogs. *Indian J Exp Biol* 1997;**35**:244-9.

131. Sharma SK, Khanna M, Sharma S. Effect of corticosteroid treatment on various serological and bronchoalveolar lavage abnormalities in patients with sarcoidosis. *Indian J Med Res* 1995;**101**:207-12.

132. Sembulingam K, Semulingam P, Namasivayam. Effect of *Ocimum sanctum* Linn on noise induced changes in plasma corticosterone level. *Indian J Physiol Pharmacol* 1997;**41**: 139-43.

133. Udupa SL, Udupa AL, Kulkarni DR. A comparative study on the effect of some indigenous drugs on normal and steroid-depressed healing. *Fitoterapia* 1998;**69**:507-10.

134. Shaji AV, Kulkarni SK. Central nervous system depressant activities of melatonin in rats and mice. *Indian J Exp Biol* 1998;**36**:257-63.

135. Karimungi MG, Joshi BN, Saibaba P. Influence of melatonin on blood morphology in male albino mice. *Indian J Exp Biol* 1996;**34**:758-63.

136. Dhanaraj, Joshi BN. Effect of long-term administration of melatonin on eyes and Harderian glands in albino rat. *Indian J Exp Biol* 1995;**33**:664-7.

137. Batmanabane M, Ramesh KG. Effect of exogenous melatonin on the onset of puberty in female albino rats. *The Anatomical Record* 1996;**245**:519-24.

PHARMACOLOGY OF MEDICINAL PLANTS AND NATURAL PRODUCTS

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1. Introduction

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last 5 year period. The present review aims to compile data generated through the research activity using modern scientific approaches and innovative scientific tools in last 5 year period *i.e.* 1994-1998.

To facilitate the readers to look at their areas of interest more easily, the data in the present review have been organised in various sections according to pharmacological activities. Two of these sections deserve special mention - one on nutraceutics, in which research on plants that form a part of our normal diet has been compiled irrespective of activity and the second on phytochemical studies which are associated with pharmacological activity. In the rest of the sections, the description on individual plants is followed by that on polyherbal formulations. Polyherbal formulations have been included as they are widely used, as reflected in the study by Karandikar *et al*,[1] and the data based on the studies carried out with these formulations has been published in peer-reviewed journals.

2. CNS Active Plants

The scope of CNS active Indian Medicinal Plants in therapeutics has been illustrated in a review article by Vaidya[2]. The following paragraphs focus on the further work carried out during the last 5 years.

2.1. Nootropics

Various extracts derived from the seeds of *Pongamia pinnata* (Karanj) decreased pentobarbitone sleeping time, probably by stimulation of the hepatic microsomal enzyme system[3]. Similar properties were exhibited by its roots. However, the petroleum ether extract (PEE) of the roots enhanced pentobarbitone sleeping time, probably due to CNS depression[4]. The PEE of the seed of *Pongomia pinnata* was further tested for nootropic activity in an experimental model of Alzheimer's disease (created by ibotenic acid induced lesioning of nuclear basalis magnocellularis). It reversed both, the cognitive deficits and the reduction in cholinergic markers after 2 weeks of treatment. Reversal of perturbed cholinergic function appears to be the possible mechanism[5].

The alcoholic extract of *Bacopa monniera* facilitated the acquisition, consolidation and retention of memory as seen by its effect on 3 newly acquired behavioural responses in albino rats, *viz.* foot shock motivated brightness discrimination, active conditioned avoidance and Sidman continuous avoidance responses. Further studies identified the chemical constituent, a mixture of 2 saponins designated as bacosides A & B, responsible for the facilitatory effect of *Bacopa monniera* on learning schedules. The bacosides also attenuated the retrograde amnesia produced by immobilisation induced stress, electroconvulsive shock and scopolamine and enhanced protein kinase activity and increased the protein content in the hippocampus. Phase I clinical studies have confirmed the safety of the bacosides in healthy male volunteers at both single and multiple doses administered over a period of 4 weeks[6].

The acetone soluble fraction of petroleum ether extract of *Lawsonia inermis* (Mehendi) leaves showed significant nootropic effect on the elevated plus maze and passive shock avoidance paradigms. The extract also potentiated clonidine induced hypothermia and decreased lithium induced head twitches. This indicates that it affects 5HT and noradrenaline mediated behaviour. It had no effect on haloperidol induced catalepsy, thus showing no effect on dopamine mediated behaviour[7].

A lot of attention has been focused on the effect of BR-16A (Mentat), a polyherbal formulation, on learning and memory. It has been shown to augment acquisition and retention of learning in normal rats, as well as in states of cognitive deficits induced by variety of insults including pre-natal undernutrition, post-natal environmental impoverishment, sodium nitrite hypoxia, aluminium, increasing age and electroconvulsive shock (ECS) induced antero-grade and retro-grade amnesia[8-10]. Ramteke,*et al,*[11] demonstrated that administration of BR-16A to slow learning rats, improved learning (both speed and magnitude) on the Hebb Williams complex maze as compared to control. The results indicated that BR-16, like piracetam, could facilitate learning and memory, and could be categorized as a nootropic agent.

Bhardwaj and Srivastava[12] have shown that Mentat (designated by them as CIHP III), significantly improved the avoidance learning during endurance performance in rats when tested on the Runimex, a circular runaway. The number of stimuli *i.e.* electrical shocks required to induce learning were considerably reduced in the treated animals.

Trasina, a polyherbal formulation, was found to exert significant nootropic effect following 21 days therapy in 2 experimental models of Alzheimer's disease. These models were induced in rats by either injecting colchicine (15 µg/rat) intra-cerebroventricularly (*i.c.v.*) or lesioning of nucleus basalis magnocellularis by ibotenic acid (10 µg/rat). Trasina improved both memory and levels of various cholinergic markers like acetylcholine concentration, choline acetyl transferase activity and muscarinic cholinergic receptor binding in frontal cortex and hippocampus of rat brain. Thus its nootropic effect may be attributed to correction of cholinergic dysfunction[13].

Pre-treatment with Memorin (200 mg/day/kg), another herbal formulation, was found to attenuate electroconvulsive shock induced retrograde amnesia in rats when tested for passive avoidance learning paradigms in a shuttle box[14].

2.2. Psychoactives

The leaf extract of *Azadirachta indica* (Neem) exhibited anxiolytic effects comparable to diazepam at low doses (10-200 mg/kg), when tested in rats. Higher doses (>400 mg/kg) however, did not show anxiolytic activity[15].

Ethanol extract and cold aqueous infusion of *Vitex leucoxylon* (Nirnochi in Tamil) leaf depressed spontaneous motor activity, antagonised d-amphetamine induced sterotypy and oxotremorine induced tremors and shortened the duration of immobility in the behavioural 'despair' test in mice[16].

Methanolic extract of rhizomes of *Nelumbo nucifera* (Kamal) was found to cause significant reduction in spontaneous activity, decrease in the exploratory behavioural pattern by the head dip andY maze tests, muscle relaxant activity and potentiation of pentobarbitone induced sleeping time[17].

Mitra, *et al,* [18] have shown that the root powder of *Panax ginseng* did not affect pentobarbitone sleep time or spontaneous motor activity. Although it potentiated amphetamine induced increase in motility, it attenuated the other effects of amphetamine, *viz.* stereotypy and lethality in aggressive mice. Haloperidol catalepsy was potentiated while the behavioural responses of 5-hydroxytryptophan and l-dopa were both attenuated. It exhibited significant aggression-inhibiting effect in doses that had no effect on spontaneous movements. The results have been discussed on the basis of interaction of *Panax ginseng* with the functioning of various neurotransmitters.

All extracts [petroleum ether (PEE), benzene(BE), chloroform (CE), acetone (AE) and ethanol (EE)] of the leaf of *Abies pindrow* Royle (Silver fir) showed potentiation of pentobarbitone sleeping time *i.e.* CNS depressant effect. The PEE, BE, CE and AE (highest efficacy) showed significant anti-depressant activity. On the other hand, EE was found to potentiate immobility, suggesting that this fraction is devoid of anti-depressant effect [19].

Ginkgolic acid conjugates (GAC) (6-alkylsalicylates, namely n-tridecyl-, n-pentadecyl-, n-heptadecyl-, n-pentadecenyl- and n-heptadecenylsalicylates) isolated from the leaves of Indian *Ginkgo biloba* Linn., a PAF antagonist, showed consistent and significant anxiolytic activity. By contrast, EGb 761 and Ginkocer, 2 conjugates which are devoid of GAC, did not evoke significant activity. EGb 761 was found to increase rearing and decrease immobility time only in open field behaviour. This effect may be due to weak anti-anxiety activity[20].

Administration of BR-16A for 7 days induced dose related anxiolytic effects as assessed by paradigms like the open field and elevated plus maze tests in mice and the social interaction and Vogel's drink conflict tests in rats. It attenuated the increase in rat brain tribulin levels, a putative endocoid anxiety marker footshock - induced aggressive behaviour in paired rats but failed to affect clonidine-induced automutilative behaviour. Reduction in swim stress induced immobility in Porsolt's behavioural despair test, reduction in escape failures concomitant with an increase in avoidance response in the learned helplessness test and attenuation of muricidal behaviour in rats demonstrated that it possesses anti-depressant properties[21].

2.3. Agents attenuating dependence

Chronic treatment with the root extract of *Withania somnifera* (Ashwagandha) attenuated the development of tolerance and also the development of dependence to morphine in mice. By itself, *Withania somnifera* showed no analgesic effect[22].

2.4. Anticonvulsants

In a study carried out by Manocha, *et al* [23], *Ginkgo biloba* decreased the protective effect of sodium valproate and carbamazepine against picrotoxin as well as strychnine induced convulsions in mice. Further studies showed that pre-treatment with *Ginkgo biloba* extract potentiated the convulsions produced by picrotoxin and strychnine, indicating the involvement of GABAergic system and chloride channels (for picrotoxin) and modulation of action of the glycine neurotransmitter (for strychnine) by *Ginkgo biloba* [24].

Panax ginseng was shown to have no anticonvulsant action, nor did it potentiate the anticonvulsant effects of phenobarbitone and diazepam[18].

Chronic administration of BR-16A was found to protect against pentylenetetrazole (PTZ) induced kindling in mice, demonstrating the role of GABA receptor in PTZ induced kindling and protection by BR-16A by its interaction with these receptors[25].

Caffeine intake has been shown to increase the plasma half- life (2-fold) and reduce the bioavailability by 32% of carbamazepine in normal human volunteers. No interaction was seen with sodium valproate. These results indicate the need for restriction of xanthine/caffeine consumption in patients on carbamazepine therapy[26].

2.5. Sedatives

The non-polar fractions of the leaf of *Vernonia* species (Sahadevi) *viz. Vernonia lasiopus* and *Vernonia galamensis* have been shown to have sedative properties in rats[27].

2.6. Analgesics

Gossypin , a bioflavonoid from the yellow petals of *Hibiscus vitifolius* (Bhasadwaji), has been shown to have anti-nociceptive activity, similar to morphine and involving multineurotransmitter systems, mainly the cholinergic and GABAergic neurotransmitter pathways. Gossypin pre-treatment significantly decreased the development of acute tolerance to morphine induced anti-nociception (acetic acid induced writhing assay). Thus, it is a potential candidate for clinical trials as an analgesic with the advantages of lack of tolerance and dependence liability[28].

Suppression of acetic acid writhing was seen with both the ethanol extract and cold aqueous infusion of *Vitex leucoxylon* [16].

Azadirachta indica showed analgesic properties in mice. Pre-treatment with the opioid antagonist, naloxone and central noradrenaline depletor, DSP-4, attenuated the analgesia whereas the serotonin synthesis inhibitor, PCPA, potentiated the same, suggesting that both central and peripheral mechanisms and complex neural pathways (opioid and non-opioid *i.e.* monoaminergic) may be involved in this effect[29].

Cerpegin, a novel furopyridine alkaloid isolated from Ceropegia *juncea Roxb.* (Bhutumbi), has shown an analgesic effect (not involving the opioid pathway) against acetic acid induced writhing in mice[30]. Using the same model in rats, significant analgesic activity was detected in leaf and seed of *Vernonia lasiopus* and *Vernonia galamensis* [27] and alcoholic extract of *Ochna obtusata* (Kanakchampak) stem bark[31]. *Panax ginseng* exhibited anti-nociceptive activity and potentiated the anti-nociceptive activity of both pentazocine and aspirin[18].

The PEE, BE and EE of the roots of *Pongamia pinnata* showed significant analgesic effect in the tail flick test[4]. The PEE and direct EE of the seeds also showed[3] significant analgesic activity at doses higher than 100 mg/ kg.

Different extracts of *Abies pindrow* Royle leaf (PEE,BE,CE,AE and EE) showed significant analgesic effect in the hot wire induced tail flick response in rats. Possible mechanism of action could be its inhibitory effect on PAF and prostaglandins as this plant contains phyto-constituents such as flavonoids and terpenoids[19].

Alcoholic extract of the roots of *Clerodendron serratum* (Bharanji) showed significant analgesic activity in mice[32].

2.7. Anti-inflammatory agents

The ethanolic extract of the leaf of *Vitex leucoxylon* showed significant inhibition of carrageenin paw oedema and granulation tissue formation in rats[16].

The aqueous suspension of dried latex of *Calotropis procera* (Arka) showed anti-inflammatory property when tested in the carrageenin and formalin induced rat paw oedema models[33].

The roots and leaves of *Butea frondosa* (Palash) were evaluated for ocular anti-inflammatory activity in rabbits. The results showed that the gel formulation of *Butea frondosa* leaves, prepared using a commercially available, pluronic F-127, reduced the intra-ocular pressure, decreased leucocytosis and miosis and was comparable to flubiproten gel[34].

The triglyceride fraction of oil of *Ocimum sanctum* (Tulsi) offered higher protection against carrageenin induced paw oedema in rats and acetic acid induced writhing in mice, as compared to the fixed oil[35]. Fixed oil of *Ocimum sanctum* and linolenic acid were found to possess significant anti-inflammatory activity against PGE_2, leukotriene and arachidonic acid induced paw oedema. The anti-inflammatory activity of linolenic acid present in the fixed oil of *Ocimum sanctum* was probably due to blockade of both, the cyclo-oxygenase and lipo-oxygenase pathways of arachidonic acid metabolism[36].

Alcoholic extract of *Ochna obtusata* stem bark demonstrated potent anti-inflammatory effects in the rat paw oedema and cotton pellet granuloma models[31].

All extracts of the root of *Pongamia pinnata* showed significant anti-inflammatory activity (compared to phenylbutazone) in carrageenin and PGE_1 induced oedema models. Possible mechanism of action could be prostaglandin inhibition, especially by EE and AE. The BE was effective in carrageenin but not the PGE_1 model of inflammation. The anti-inflammatory property appears to reside mainly in the intermediate polar constituents and not in lipophilic or extremely polar constituents[37].

The PEE and CE of the seeds of *Pongamia pinnata* showed potent acute anti-inflammatory effect whereas the aqueous suspension showed pro-inflammatory effects. Further studies have shown that maximum anti-inflammatory effect was seen in the bradykinin induced oedema model with the direct EE[38]. Possible mechanism of action could be inhibition of prostaglandin synthesis and decreased capillary permeability. PEE and AE inhibited histamine and 5- hydroxytryptamine induced inflammation probably by their lipophilic constituents preventing the early stages of inflammation. However, the fractions were not effective against Freund's adjuvant arthritic model. The latter finding indicates that the plant may not be effective in rheumatoid arthritis.

All extracts of *Abies pindrow* Royle leaf showed anti-inflammatory effect in various animal models of inflammation such as carrageenin induced paw oedema, granuloma pouch and Freund's adjuvant arthritis. Chemical analysis indicated the presence of glycosides and steroids in the PEE and BE and terpenoids and flavonoids in the AE and EE. Flavonoids and terpenoids are polar substances effective in acute inflammation whereas glycosides and steroids are non-polar substances effective in chronic inflammation[39].

The methanolic extracts of the flowers of *Michelia champaca* Linn. (Champaka), *Ixora brachiata* Roxb (Rasna) and *Rhynchosia cana* Willd were found to possess significant anti-inflammatory activity against cotton pellet induced subacute inflammation in rats. The latter 2 drugs showed higher activity as compared to *Michelia champaca*. They also reduced the protein content, acid phosphatase, glutamate pyruvate transaminase and glutamate oxaloacetate transaminase activities in the liver and serum. These properties are probably due to the presence of flavonoids in the flowers of these plants[40].

The methanolic extract of the aerial part of *Sida rhombifolia* (Atibala) showed significant oedema suppressant activity in the carrageenin induced paw oedema model in rats. Probable mechanism of action may be due to its inhibitory effects on release of mediators of inflammation such as histamine, 5-hydroxytryptamine, bradykinin *etc*[41].

Gmelina asiatica (Gopabhadra) root powder was effective in reducing the oedema in the carrageenin-induced rat paw oedema model of acute inflammation. When tested against the cotton pellet granuloma model of chronic inflammation, it not only reduced the weight of the granuloma but also the lipid peroxide content of granuloma exudate and liver and gamma-glutamyl transpeptidase in the granuloma. It also normalised serum albumin and serum acid and alkaline phosphatase levels. Probable mechanism of its anti-inflammatory effect may be its anti-proliferative, anti-oxidative and lysosomal membrane stabilising effects[42].

Studies have shown that, the methanol extract of *Nelumbo nucifera* rhizome as well as the steroidal triterpenoid isolated from it (betulinic acid), possessed significant anti-inflammatory activity when evaluated in the carrageenin and 5-hydroxytryptamine induced rat paw edema models. The effects produced were comparable to that of phenylbutazone and dexamethasone[43].

The water soluble part of the alcoholic extract of *Azadirachta indica* exerted significant anti-inflammatory activity in the cotton pellet granuloma assay in rats. Levels of various biochemical parameters studied in cotton pellet exudate were also found to be decreased *viz.* DNA, RNA, lipid peroxide, acid phosphatase and alkaline phosphatase suggesting the mechanism for the anti-inflammatory effect of *Azadirachta indica*[44].

Alcoholic extract of the roots of *Clerodendron serratum* showed significant anti-inflammatory activity in the carrageenin induced paw oedema and cotton pellet granuloma models in rats[32].

The aqueous extract of *Gymnema sylvestre* leaves showed significant anti-inflammatory activity in the carrageenin induced rat paw oedema and mouse peritoneal ascitis models. It, however, did not inhibit granuloma formation and related biochemical indices, such as hydroxyproline and collagen, (as seen in the pith granuloma model) thus indicating that it did not interfere in the normal healing process. In addition, the extract did not affect the integrity of the gastric mucosa, even at high doses, thus appearing to be a less gastrotoxic anti-inflammatory agent as compared to other non-steroidal anti-inflammatory agents[45].

Sandhika, an Ayurvedic drug used in the treatment of rheumatoid arthritis showed significant anti-inflammatory activity when tested against carrageenin induced paw oedema and cotton pellet granuloma. Possible mechanism of action could be by free radical scavenging activity[46].

Treatment with Ease, a polyherbal formulation, significantly reduced Freund's adjuvant-induced non-established and established arthritis in rats. *In vitro* too, it provided significant protection against protein denaturation and RBC membrane damage and exhibited significant proteinase inhibitory action, thus indicating its possible use as an anti-arthritic[47].

Jigrine, another polyherbal formulation, exhibited anti-inflammatory activity against carrageenin induced acute inflammation but not against cotton pellet granuloma (subacute inflammation). Effect on biochemical parameters suggested that the mechanism of its anti-inflammatory effect could be in its antioxidant and membrane stabilising effect[48].

2.8. Antipyretics

The ethanolic extracts of *Ailanthus excelsa* (Mahanimba), *Toddalia asiatica* (Kanchana) and *Araucaria bidwilli* (Monkey puzzle) showed moderate to significant degree of antipyretic activity in an experimental rat model of 20% yeast suspension induced hyperthermia[49]. *Andrographis elongata* showed more potent antipyretic activity when compared to *Andrographis paniculata* (Kalmegha)[50]. Methanolic extract of rhizome of *Nelumbo nucifera* produced a significant dose dependent lowering of body temperature in normal rats and antipyretic effect in pyretic rats [51]. *Rhynchosia cana* showed significant antipyretic activity in rats[40]. Alcoholic extract of the roots of *Clerodendron serratum* showed significant antipyretic activity following typhoid TAB vaccination in rabbits[32].

The antipyretic activity of TBR-002, a herbal formulation, was found to be almost equal to paracetamol in a rat model of pyrexia induced by subcutaneous injection of 15% yeast suspension[50].

As against these antipyretic plants, *Panax ginseng* showed hyperthermic effect and attenuated the hypothermic response of reserpine and 5-HTP induced hyperthermia in animals[18].

2.9. Neurotransmitter modulation

Investigation of the neurochemical effects of different fusarial toxins elaborated from *Fusarium moniliform* and *Fusarium orysporum* showed that *Fusarium moniliform* had irreversible and nonspecific MAO inhibitory activity comparable to nialamide[52].

Studies on the effect of BR-16A on adrenergic and dopaminergic functioning in rats showed that it did not interfere with α_2 -adrenergic and dopamine autoreceptor functioning. However, its effects on mobility in the open field test following challenge with clonidine or apomorphine showed that it enhanced dopamine post-synaptic receptor activity[53].

3. Plants modulating autonomic and autacoid activity

Over the years, the trend to evaluate agents modulating autonomic and autacoid activity is declining. However, importance of using certain methodologies like isolated tissue experiments to gather preliminary data regarding interaction of a plant product with host receptor systems remains unquestionable. Following are some of the attempts in this direction.

The effects of stem extract of *Cuscuta reflexa* (Amarvalli) resembled those of acetylcholine when tested on isolated rabbit ileum, frog rectus abdominis and heart and these effects were blocked by atropine. Effect of the extract on isolated frog rectus abdominis muscle was blocked by pancuronium and potentiated by neostigmine[54].

Hot water extract of *Camellia sinensis* (Green tea leaf extract, GTE) had a facilitatory effect at lower concentrations and a paralytic effect at higher concentrations on skeletomotor function but did not have any effect on direct twitch responses or on acetylcholine and KCl induced contractures of denervated rat diaphragm. In addition it antagonised the submaximal paralytic effect of d-tubocurarine and decamethonium. The effects of GTE were nullified in the presence of magnesium chloride. Since nifedipine reduced GTE-induced facilitation as well as inhibition of twitch responses, it has been suggested that GTE might act on Ca^{2+} channels at the skeletomotor junction. The effect of crude polyphenol of GTE on neuromuscular junctions was found to be similar to that of GTE suggesting that the crude polyphenol content of GTE was the active constituent responsible for its effect on neuromuscular junction[55].

Beta-adrenoreceptor mediated tracheal relaxation or the decreased responsiveness of the tracheal smooth muscle induced by down-regulation of receptors with terbutaline in guinea pigs was unaffected by Abana, a herbomineral formulation. This is probably due to lack of Abana's effects on ß-receptors of the airway. However, pretreatment with Abana increased potassium chloride-induced contractions and increased the sensitivity to the relaxant effects of isoprenaline, terbutaline and aminophylline following such contractions, probably by enhancing membrane permeability to calcium ions[56].

4. CVS active plants

Research in cardiovascular pharmacology in the past few years has been mainly focused on agents with hypolipidaemic properties and plant drugs are no exception. This section describes various agents that have been evaluated for their effect on the cardiovascular system.

4.1. Anticoagulant

The petroleum ether and methanolic extracts of the leaf and oleoresin of *Araucaria bidwillii* showed marginal delaying effect on bleeding and clotting times at 1 hour interval in rabbits when tested using Wright's and Dukes capillary tube method[57].

4.2. Hypolipidaemics

The ethanol extract and cold aqueous infusion of *Vitex leucoxylon* leaf lowered serum total cholesterol levels in mice[16].

Gugulipid (an active principle of *Commiphera mukul*) is an agent that has been widely investigated for its hypolipidaemic activity. Co-administration of gugulipid with propranolol or diltiazem in normal volunteers was found to decrease the bioavailability of both drugs[58].

Dried flowers of *Adenocalymma alliaceum* when fed at 2% level for 6 weeks to hypercholesterolaemic rats, lowered serum cholesterol levels significantly, lowering the absorption of dietary cholesterol from the intestines[59].

Semecarpus anacardium (Bhallatak, nut shell) extract also exhibited hypocholesterolemic action and prevented cholesterol induced atheroma in hypercholesterolaemic rabbits[60]. Similarly, *Terminalia belerica* (Bibhitak) reduced the levels of lipids in experimentally induced hypercholesterolaemia in rabbits. There was also a significant decrease in liver and heart lipids[61].

Administration of ethanolic extract (50% v/v) of *Plumbago zeylanica* (Chitrak) root, alone and in combination with vitamin E, significantly reduced serum total cholesterol, LDL cholesterol and triglyceride levels in experimentally induced hyperlipidaemic rabbits[62]. However, Dwivedi[63] pointed out that the ethanolic extract of *Plumbago zeylanica* root alone and with vitamin E lowered HDL cholesterol levels as well. Hence, he has advised caution about its use in patients and confirmation of these findings through larger sample size studies.

Administration of cell culture extract of *Hemidesmus indicus* (Sariva) in rats also receiving an atherogenic diet prevented hypercholesterolaemia[64].

4.3. Anti-hypertensives

Preparation of the whole plant of *Phyllanthus amarus* (Bhuiamalaki) was administered to 9 mild hypertensive subjects for 10 days. The results suggest that it is a potential diuretic, hypotensive and hypoglycemic drug for humans[65].

Hydroalcoholic leaf extract of *Azadirachta indica* caused a dose-dependent hypotensive effect. It did not alter the force of contraction or heart rate at low doses in isolated frog heart, but caused a temporary cardiac arrest in diastolic at high doses[66].

The petroleum ether extract of *Abies pindrow* leaf showed significant hypotensive effect in anaesthetized dogs[19].

Treatment with Abana, a polyherbal formulation, in normotensive rats produced significant lowering of blood pressure, enhancement of vaso-pressor responses to low dose of noradrenaline (with no effect on dopamine β hydroxylase activity) and no effect on the vaso-depressor responses of acetylcholine and isoprenaline. It, however, protected against ethinyl oestradiol induced hypertension and increased dopamine β hydroxylase activity in these hypertensive animals, thus suggesting that it produces effects against ethinyl oestradiol induced hypertension by its sympatholytic property[67].

4.4. ACE (angiotensin converting enzyme) inhibitors

Seventy- five species of traditional medicinal plants belonging to 42 families have been investigated for their ability to inhibit the angiotensin converting enzyme. Of these, 4 species were found to possess a high ACE inhibiting ability and were low in their tannin content[68].

4.5. Cardio-protectives

Rutin, a flavonoid, obtained from the plant, *Sophora japonica*, markedly reduced the infarct size and prevented the loss of the 'R' wave in anaesthetized rats subjected to coronary artery ligation. It, however, had no effect on heart rate and systolic blood pressure. It also reduced the ligation-induced increase in serum malonyldialdehyde levels and prevented the loss of glutathione peroxidase activity. Rutin inhibited, *in vitro*, luminol-induced chemiluminescence of rat PMN's, thus indicating that its beneficial effect is probably due to its ability to impair the generation of reactive oxygen species[69].

4.6. Positive ionotropics

Vaidya[70] in his editorial, had raised the issue on the controversies that exist regarding ionotropic actions of *Terminalia arjuna* (Arjuna). Though there are studies proving positive ionotropic effects of this plant, some scientists have observed negative ionotropic and chronotropic effects as well. Hence, he had advised more detailed experimental and clinical studies on the plant and its active principle. Data of placebo-controlled clinical studies carried out subsequently with this plant is presented below.

When the bark extract of *Terminalia arjuna* was compared to placebo in 12 patients with refractory CCF in a phase II clinical trial, it was observed that treatment with *Terminalia arjuna* was associated with an improvement in symptoms and signs of heart failure, improvement in the NYHA class (from IV & III), decrease in echo-left ventricular end-diastolic and end-systolic volumes indices, increase in the left ventricular stroke volume index and increase in left ventricular ejection fraction at the end of 2 weeks. Long term therapy (*i.e.* 24 months) too showed continued improvement in the signs and symptoms, effort tolerance and NYHA class among the patients[71].

Dwivedi and Jauhari[72] studied the effects of bark stem powder of *Terminalia arjuna*, as compared to placebo, on angina pectoris, CCF and left ventricular mass in 12 patients of myocardial infarction with angina and/or ischaemic cardiomyopathy. Their findings indicate the potential of *Terminalia arjuna* improving left ventricular ejection fraction and reducing left ventricular mass in coronary artery disease.

5. Plants acting on respiratory system

Methanolic extracts of *Drymaria cordata* willd and *Leucas lavandulaefolia* (Dronapushpi) were investigated for their effects on a cough model induced by sulfur dioxide gas in mice. Both exhibited significant anti-tussive activity, comparable to that of codeine phosphate and increasing concentrations showed better inhibition of cough[73,74].

Solanum xanthocarpum (Kantakari) and *Solanum trilobatum* (Alarka), the plants mentioned in Siddha, have been shown to improve various parameters of pulmonary function (FVC, FEV_1, PEFR & FEF25-75%) in asthmatic subjects with mild-moderate asthma[75].

6. Anti-allergic plants

Ethanolic extract of *Vitex negundo* (Nirgundi) of was found to inhibit immunologically induced degranulation of mast cells better than that with compound 40/80. It also inhibited be oedema during active paw anaphylaxis in mice[76]. Nair and Saraf [77] further studied their effects on mediator release and smooth muscle contractions of sensitized and non-sensitized guinea pig trachea using antigen and compound 48/80 respectively.The extract significantly inhibited both the initial and later sustained phases of tracheal contractions. The initial phase was primarily due to histamine release which was blocked by the extract (confirmed in guinea pig ileal studies). The latter phase was due to release of lipid mediators from arachidonic acid. Inhibition of the latter phase may be secondary to inhibition of arachidonic acid by the ethanolic extract.

Arbortristoside A & C, derived from the alcoholic extract of the seeds of *Nyctanthus arbortristis* (Parijat)[78], two diterpenes, andrographolide and neoadrographolide, isolated from *Andrographis paniculata*[79], and Himachalol, a sesquiterpene alcohol, derived from the hexane soluble extract of the wood of *Cedrus deodara* (deodar) [80], were found to possess significant anti-allergic activity comparable to disodium cromoglycate when tested in the experimental models of passive cutaneous anaphylaxis and mast cell degranulation in rats.

Similarly, hot aqueous extract of the bark of *Albizzia lebbeck* (Shirish) was found to possess anti-allergic properties in experimental model of passive cutaneous anaphylaxis and mast cell stabilization activity[81].

7. Hypoglycemic plants

A preparation of the whole plant of *Phyllanthus amarus* was found to have hypoglycemic effects in 9 human subjects, 4 of whom were diabetics[65].

In vitro studies carried out by Rizvi *et al*,[82] have shown that epicatechin, an active constituent of *Pterocarpus marsupium* (Vijaysar)exerted a protective effect on erythrocyte osmotic fragility, similar to insulin, but by a different mechanism of action.

In streptozotocin induced diabetic rats, of the 3 important phenolic constituents of the heartwood of *Pterocarpus marsupium* (*viz.*pterosupin, marsupin and pterostilbene) marsupin and pterostilbene significantly lowered the blood glucose levels and the effects were comparable to metformin[83]. The hypoglycemic efficacy of *Pterocarpus marsupium* has been further evaluated in a multicentric (4 centres) flexible-dose open trial in newly-diagnosed patients of non-insulin-dependent diabetes mellitus[84]. Control of blood glucose (both fasting and post-prandial levels) was attained in 67 of 97 patients (69%) studied in 12 weeks and the optimum dose was 2 g of the extract. HbA_1c values also decreased significantly. No significant change was observed in the mean levels of lipids.

The chloroform eluted fraction of the petroleum ether extract of the root bark of *Salacia oblonga* Wall (Ponkoranti) and a fluorescent compound separated from it (by TLC) demonstrated hypoglycemic potency in rats when compared to tolbutamide[85].

The alcoholic extract of *Inula racemosa* (Pushkarmula) lowered blood glucose and enhanced liver glycogen in rats. However, there was no increase in plasma insulin levels nor an increase in the degree of degranulation of beta cells of pancreas. Its action may be at the peripheral level by potentiating insulin sensitivity[86].

Hot water extract of *Camellia sinensis* (Black tea leaf) significantly reduced the blood glucose level and was found to possess both preventive and curative effects in streptozotocin induced diabetic rats[87].

The leaf extract of *Azadirachta indica* had no effect *per se*, on the peripheral utilization of glucose (determined by intravenous glucose tolerance tests) and on hepatic glycogen in normal and streptozotocin induced diabetic rats. However, it blocked the effects of epinephrine on glucose metabolism and reduction in peripheral glucose utilization in diabetic rats and to some extent in normal rats, indicative of an anti-hyperglycemic potential of the plant[88].

Leaf extract of *Aegle marmelos* (Bilva) was found to significantly reverse the raised K_m values, but not V_{max} values of the enzyme malate dehydrogenase, an important enzyme in glucose metabolism, in streptozotocin induced diabetic rats. Alteration in the qualitative and quantitative nature of the enzyme has been suggested to contribute to the pathological state of diabetes. The leaf extract was also effective in restoring blood glucose and body weight to normal values[89]. In another study[90], leaf extract of *Aegle marmelos* significantly reversed the altered (histological and ultrastructural) parameters in tissues of streptozotocin induced diabetic rats seen by light and electron microscopy to near normal and improved the functional state of pancreatic beta cells. The hypoglycemic effects of this plant drug thus appears to be mediated through regeneration of damaged pancreas.

Oral administration of the methanolic extract (but not the water extract) of aerial parts of *Artemisia pallens* (Daman) led to significant blood glucose lowering in glucose fed hyperglycemic and alloxan induced diabetic rats. Increased peripheral utilisation of glucose is probably the mechanism responsible. Inhibition of renal proximal tubular reabsorption of glucose may also contribute[91]. Saxena *et al* [92] compared the effects of mode of action of 3 structurally different hypoglycemic agents, tolbutamide, centpiperalon and a swerchirin- containing fraction (SW1) from the plant *Swertia chirata* (Chirayata) in normal and streptozotocin induced mild and severe diabetes in rats. Except in rats with severe pancreatic damage, SW1 showed better blood glucose lowering effect compared to tolbutamide.

Ocimum album (Holy basil) leaves significantly decreased the fasting and post-prandial blood glucose levels in patients with NIDDM in a randomized, placebo-controlled, crossover, single blind trial[93]. Administration of *Ocimum sanctum* leaf powder to normal and diabetic rats for a period of one month resulted in a significant reduction in fasting blood sugar, uronic acid, total amino acids, total cholesterol, triglyceride, phospholipids and total lipids. Total cholesterol, triglyceride and total lipids were significantly lowered in the liver, kidney and heart. They indicate the hypoglycemic and hypolipidemic effect of *Ocimum sanctum* in diabetic rats[94].

Chronic administration of *Prunus amygdalus* (Almond) seeds and its proportionate fractions *viz.* defatted seed and oil to rabbits demonstrated a definite hypoglycemic effect. The active factor seems to be a non-oil fraction which is only partly soluble in ethyl ether[95].

Significant hypoglycemic effect was observed with 1500 mg/kg dose of juice of leaves of *Lantana camara* in rats[96].

The protective effect of *Capparis decidua* (Karir) powder on oxidative stress and diabetes in alloxan induced diabetic rats has been evaluated. The data indicate that *Capparis decidua* may have a potential use as an anti-diabetic agent, especially in chronic cases as it helps in lowering the oxidative stress in diabetes[97].

Dubey *et al*,[98] studied the effect of D-400, a polyherbal formulation, on blood glucose, blood urea and serum creatinine in alloxan-induced diabetic rabbits. D-400 significantly prevented the rise in blood urea and serum creatinine levels at the end of 36 weeks, thus showing promise against alloxan induced renal damage. The rise in blood sugar too in the treated group was lower than the saline control. Further studies by Dhawan *et al*,[99] demonstrated that D-400, in diabetic rats, not only brought the raised blood glucose levels to within normal limits and raised the suppressed glycogen levels, but also brought towards normal, the decreased ^{14}C glucose uptake by liver slices in *in vitro* studies.

Trasina, an Ayurvedic herbal formulation, significantly reduced streptozotocin induced hyperglycemia and also attenuated streptozotocin induced decrease in superoxide dismutase activity of pancreatic islet cells in male Charles Foster rats[100].

8. Anti- and pro-fertiliy plants

The second area which has been widely worked upon in the field of endocrinology is the reproductive system.

Ethanolic extract of *Bupleurum marginatum* was found to have significant oestrogenic activity as seen by the increased uterine weight and early opening and cornification of vagina in immature rats and histological features of the uterus[101].

Praneem vilci, a highly purified oil of *Azadirachta indica* seed, was found to be safe when administered as a single intra-uterine instillation in 18 healthy tubectomised women. No untoward effects were observed. The menstrual pattern and ovulatory status remained unaltered and the endometrial biopsy was normal. In 10 of the above women who had also received the HSD-hCG vaccine, co-administration of Praneem vilci did not prevent the antibody response to HSD-hCG vaccine[102]. The leaves of *Azadirachta indica* were found to have a reversible, anti-androgenic properties in male rats[103].

Ethanolic extract of *Trichopus zeylanicum* (Arogyapacha in Tamil) leaf, when administered to male mice was found to stimulate sexual behaviour as evidenced by an increase in number of mounts and mating performance. Chronic administration of the drug was more effective than a single dose. The pups fathered by drug treated mice were also found to be normal with respect to foetal growth, litter size and sex ratio[104].

Benzene extract of *Hibiscus rosea* sinensis (Jaswand) flowers showed differing results when administered to adult and immature mice. In the adults, it resulted in an irregular estrous cycle with prolonged estrous and metaestrous. An increase in the atretic follicles and absence of corpora lutea indicated the antiovulatory effect of the extract. However, in immature mice, the extract showed oestrogenic activity as seen by the early opening of the vagina, premature cornification of vaginal epithelium and increase in uterine weight[105].

9. Plants promoting skin and bone healing

Methanolic extract of *Cissus quadrangularis* (Asthishunkala) promoted the healing process of experimentally fractured radius-ulna of dogs as evidenced by radiological and histopathological examinations[106]. The treated group also exhibited a reduction in serum calcium levels as compared to saline control animals.

Intra-dermal administration of the essential oils from the leaves of Eucalyptus hybrid and seeds of *Seseli indicum* increased cutaneous capillary permeability when tested in Evan's blue treated rabbits. This effect may be beneficial in their probable wound healing activity[107].

Topical application of aqueous extract of latex of *Euphorbia neriifolia* (Nivadung) facilitated the healing of surgically produced cutaneous wounds in guinea pigs as evidenced by an increase in tensile strength, DNA content, epithelialisation and angiogenesis[108].

Only the aqueous extract suspension in 5% propylene glycol of *Centenella asiatica* as compared to the other extracts (*viz.* alcoholic, petroleum ether, chloroform, propylene glycol and glycosidal extract) promoted wound healing in experimentally induced open wounds on topical administration in rats as evidenced by the increase in collagen content and thickness of epithelium[109]. However, Suguna *et al*,[110] demonstrated that the alcoholic extract (oral and topical) of *Centenella asiatica* improved the rate of wound healing in rats. Sunilkumar *et al*,[111] showed that topical administration of the aqueous extract increased cellular proliferation, promoted collagen synthesis at the wound site as evidenced by the increase in DNA, protein and collagen content of granulartion tissue and in tensile strength. The treated wound epithelialised faster and the rate of wound contraction was higher as compared to control. Among the various formulations (ointment,cream and gel) of the aqueous extract, the process of healing was better with the gel formulation.

The extracts of four plants *i.e.* the leaves of *Aloe vera*, root and root bark of *Aegle marmelos* and *Moringa oleifera* and leaves of *Tridax procumbes* were found to promote wound healing in both normal and immunocompromised (steroid treated) rats in a space wound model. The plants increased not only lysyl oxidase activity but also, protein and nucleic acid content in the granuloma tissue thus indicating that the plants probably exert their action at the cellular (nuclear) level. The plants also increased the tensile strength of the granuloma tissue probably as a result of the increase in the glycosaminoglycan content. Thus the plants not only hastened normal healing, but also reversed steroid depressed healing[112].

Sunder vati, an Ayurvedic formulation was effective in the treatment of acne vulgaris as seen by the significant reduction in lesion count in patients with this condition[113].

10. Plants acting on genito-urinary system

Ethanolic extract of *Ammannia baccifera* (Bhatjambol) was found to be effective in reducing the formation of urinary stones (prophylactic) as well as dissolving pre-formed ones (curative) that were induced by implantation of zinc discs in the urinary bladders of rats. The stones formed were mainly of magnesium ammonium phosphate with traces of calcium oxalate. Treatment with *Ammannia baccifera* also significantly reduced calcium and magnesium levels[114].

Lupeol and a number of its derivatives derived from *Crateva nurvala* (Varun) were found to possess significant anti-hyperoxaluric and anti-hypercalciuric activity when tested in rats against hydroxyproline induced hyperoxaluria and calciurial[115].

11. Gastro-intestinal pro- and anti-kinetic plants

The methanolic extract of rhizomes of *Nelumbo nucifera* showed significant inhibitory activity against castor oil induced diarrhoea and PGE_2 induced entero-pooling in rats. It also showed significant reduction of gastro-intestinal motility in rats, thus indicating its efficacy as an anti-diarrhoeal agent[116].

Three different dosage formulations (aqueous extract, dry powder and incinerated powder) of *Emblica officinalis* (Amalki) were evaluated for their effect on gastro-intestinal motility at different dose levels (210,420 and 840 mg/kg) in mice. The dry powder and the aqueous extract showed pro-kinetic effect at all the 3 dose levels. The incinerated powder at lower doses, showed pro-kinetic effect, whereas at higher doses, it decreased gastro-intestinal motility[117].

12. Cytoprotective plants

12.1. Ulcero- protectives

12.1.1. Gastric and duodenal ulcers

The alcoholic, oleoresin and petroleum ether extracts of leaf of *Araucaria bidwillii* showed moderate degree of ulcero-protective activity in pylorus ligated rat model of gastric ulceration[58].

Leaf and seed extracts of *Vernonia lasiopus* and *Vernonia galamensis* had antiulcerogenic effects when tested using either hydrochloric acid or ethanol as the necrotising agent in rats[27].

The protective effect of hot water extract of black tea (*Camellia sinensis*) was demonstrate on ulcers induced in rats by various ulcerogens (NSAIDs, ethanol, reserpine, 5-HT, histamine) and by cold restraint stress 5-HT and histamine. It altered the acid and peptic activity of gastric secretion[118].

All four sitavirya plants *viz.* Satavari (*Asparagus racemosus*: fresh root juice, 1250 mg/kg), Yastimadhu (*Glycerrhiza glabra*: water decoction of root, 600 mg/kg), Kutaja (*Holorrhena antidysentrica* : water decoctions of bark, 400 mg/kg) and Aswattha (water decoctions of bark, 500 mg/kg) were were found to have ulcero-protective effects against 2 hr cold restraint stress ulcers, pylorus ligation-induced gastric and cysteamine-induced duodenal ulcers in rats. However, they were ineffective against acute aspirin-induced gastric ulcers[119].

All extracts of the seeds of *Pongamia pinnata* showed significant anti-ulcerogenic effect in fasting mice[3]. The petroleum ether(PEE), benzene and ethanolic(EE) extracts of the root of the same plant showed significant anti-ulcerogenic effect in the pylorus ligated rat ulcer model. The EE but not PEE decreased acid pepsin secretion and increased mucus secretion. PEE showed significant CNS depression as mentioned earlier, thus probably relieving stress induced ulceration[4].

Bergenin and norbergenin, two isocoumarins, isolated from the leaves and roots of *Flueggea microcarpa* and luvangetin, a pyranocoumarin isolated from the seeds of *Aegle marmelos* Correa, gave significant protection against pylorus ligation- and aspirin-induced gastric ulcers in rats and cold restraint-induced gastric ulcers in rats and guinea pigs. The gastro-protective effects of bergenin and norbergenin could be due to increased prostaglandin production as demonstrated using human colonic mucosal incubates. This mechanism was not responsible for the observed effects of luvangetin as it did not affect prostaglandin production[120].

All extracts (petroleum ether, benzene, chloroform, acetone and ethanolic) of *Abies pindrow* leaf showed ulcero-protective effect in a model of cold restraint stress because of their anti-stress effects. The extracts contained terpenoids and flavonoids which were shown to have marked inhibitory effect on PAF. Flavonoids have also been shown to increase mucus secretion, prostaglandin synthesis and blood flow[19].

Pretreatment with the aqueous extract of *Emblica officinalis* protected against ethanol- induced and cold restraint- induced gastric damage in rats (evaluated by the Evan's blue method) [117].

Cauvery 100, a polyherbal formulation, showed anti-ulcerogenic activity when tested against indomethacin induced ulcers in rats. It decreased the raised hexosamine and sialic acid levels in the ulcer towards normal, increased pepsin activity and gastrin levels and increased the uptake of titrated thymidine into the ulcer area, as compared to untreated animals[121].

Similarly, another polyherbal formulation, UL-409, demonstrated significant anti-ulcerogenic activity in three experimental studies carried out separately by Mitra *et al* [122], Vanisree *et al* [123] and Kulkarni and Goel[124].

12.1.2. Ulcerative colitis

Gupta *et al* [125] studied the protective effect of *Boswellia serrata* in patients suffering from (grade II and III) ulcerative colitis. The stool characteristics along with histopathological, scan microscopical changes in rectal biopsies and blood parameters (haemoglobin, serum iron, calcium, phosphorus, proteins, total leukocytes and eosinophils) improved following treatment with *Boswellia serrata* gum resin preparation; the results being similar to sulfasalazine.

12.2. Hepato-protectives

Two reviews have been published which cover most of the works carried out in this field. Vaidya *et al,* [126] have reviewed the experimental and clinical research work related to hepato-protective effects of various formulations available in the Indian market. Bhatt and Bhatt[127] have not only compiled the information available regarding the studies on various promising plant drugs from India but also have discussed the problems and pitfalls pertaining to this research. Some of the agents worth mentioning are as follows:

Tinospora cordifolia, a plant which has been shown to decrease fibrosis in rats, induced by CCl_4, was to significantly improved the suppressed Kupffer cell function in another rat model of chronic liver damage induced by heterologous serum. This raises the possibility that anti-fibrotic effect of *Tinospora cordifolia* is mediated through activation of kupffer cells[128]. When administered during the pre-operative period to patients with obstructive jaundice, it was found to decrease the post-operative morbidity and mortality (due to sepsis and liver cell failure). This was associated with bolstering of phagocytic and intracellular killing capacities of polymorphonuclear cells. Though *Tinospora cordifolia* was found to significantly decrease the complications associated with pre-operative biliary decompression *viz.* sepsis and immunosuppression, the prognosis was found to better when *Tinospora cordifolia* was instituted alone in preoperative period. These findings focus on the need to alter the preoperative management protocol for obstructive jaundice, by replacing the age-old procedure of biliary decompression by an immunomodulator[129]. The basis for improved prognosis appears to be the reduction in the incidence of endotoxaemia, as revealed from the studies in the rat model of cholestasis wherein *Tinospora cordifolia* was found to decrease the mortality in cholestatic rats. Earlier the therapy following cholestasis, better were the results[130].

Picroliv is a standardised preparation of irioid glycosides, picroside-1 and kutkoside obtained from *Picorrhiza kurroa.* Both picroliv and silymarin, a flavolignan component of *Silyburn marianum* seeds were shown to stimulate liver regeneration in the early stages when evaluated on partially hepatectomised rats. Both the drugs increased the levels of DNA, RNA, protein and cholesterol[131].

Picroliv administration in rats following exposure to alcohol resulted in lowering of various biochemical parameters of liver and serum that were elevated with alcohol consumption[132]. It protected against ethanol

(40%) induced toxicity in isolated rat hepatocytes[133]. I also showed a dose dependent hepatoprotective effect against oxytetracycline induced hepatic damage in rats[134]. The protective effect of picroliv against hepatic damage caused by *Plasmodium berghei* infection in *Mastomys caucha* was studied by Ramesh *et al*[135]. Treatment with this drug significantly reversed the changes in lipid metabolism induced by *Plasmodium berghei*. It reactivated the plasma and liver lipolytic enzymes, stimulated the binding of LDL with the liver receptors and enhanced the faecal excretion of bile acids, thus resulting in a return of circulatory lipoproteins towards normal.

Santra *et al*,[136] demonstrated that treatment with *Picrorrhiza kurroa* in carbon tetrachloride treated mice significantly reversed the altered serum ALT, AST, liver GSH, total thiol, glucose 6 phosphate dehydrogenase (G6PD), catalase and membrane bound Na^+/K^+ ATPase levels. Histopathological lesions of liver and lipid peroxidation were also significantly less in drug treated animals. Probable mechanism of action of *Picrorrhiza kurroa* appears to be its effect as a free radical scavenger and inhibitor of lipid peroxidation of liver plasma membrane.

The diterpenes, andrographolide, andrographiside and neoandrographolide, isolated from *Andrographis paniculata* demonstrated anti-oxidant effects in CCl_4 treated mice. Neoandrographolide was as effective as silymarin with respect to its effects on reduced glutathione, glutathione 5-transferase, glutathione peroxidase and superoxide dismutase and lipid peroxidation whereas andrographiside had mainly anti-lipoperoxidant activity[137]. Administration of the aqueous extract of *Andrographis paniculata* to mice suffering from liver damage induced by hexachlorocyclohexane (BHC) that ultimately leads to tumor formation, significantly lowered the raised enzymes (SGPT, SGOT, alkaline phosphatase) and raised the lowered protein concentration. The results confirm the hepato-protective property of *Andrographis paniculata* and suggest its probable role in delaying the hepatic tumorigenic condition[138].

Administration of Liv-52, a polyherbal formulation, significantly improved ethanol metabolism in a rat model of chronic alcohol administration[139]. It also prevented lipid peroxidation in CCl_4 induced liver damage as seen by a significant decrease in malondialdehyde content[140]. Both, Liv-52 and another formulation Kumaryasava were found to stimulate depressed hepatic enzyme activities such as hepatic arginase, cathepsin B, acid phosphatase and ribonuclease in CCl_4 induced liver damage indicating that these drugs have a protective effect on hepatic enzyme activity[141].

Liv.100 is an improvised herbal formulation of Liv52. In an *in vitro* study combination of Liv52 and Liv100 reduced the peroxidation effect of hydrogen peroxide in rat liver homogenate. The protective effect of these drugs was attributed to the enhanced supply of reduced glutathione that inhibited the deleterious process of lipid peroxidation. This suggested the anti-oxidant potential of Liv. 52 and Liv. 100[142]. Simultaneous administration of Liv-100 with the anti-tubercular drugs, INH, rifampicin and pyrazinamide showed significant protection against hepato-toxic effects of the anti-tubercular drugs in rats[143].

The hepato-protective effect of Jigrine, an Unani polypharmaceutical herbal formulation containing 14 medicinal plants, was evaluated in 3 models of hepatic damage induced by either alcohol, carbon tetrachloride or paracetamol in rats. Jigrine significantly reduced the increased serum transaminases, bilirubin, prothrombin time and liver lipid peroxide content and also improved the histopathological findings. The authors have attributed its hepato-protective effect to its anti-oxidant property[144]. Further studies carried out on Jigrine by the same group showed that it also reduced the levels of gamma-GTP, triglycerides and lipid peroxides in the liver, confirming its membrane stabilising and antioxidant properties[145].

The importance of morphological features and time of collection of raw material can be understood from a study conducted by Rawat *et al*,[146] at the National Botanical Research Institute. The effect of seasons, root thickness and dosage forms (aqueous or powder) on the hepato-protective activity of *Boerrhavia diffusa* (Punarnava) was evaluated in thioacetamide intoxicated rats. Results showed that aqueous extract of roots of diameter 1-3 cms, collected in May (Summer) exhibited marked protection as determined by assessing serum enzymes *viz*. SGOT, SGPT, ACP and ALP, but not GLDH and bilirubin. Furthermore, the studies have shown that the aqueous form of the drug has more hepato-protective activity than the powder form, probably due to better absorption of the liquid form.

A novel non-invasive parameter has been developed by Visweswaram *et al*,[147] for screening of drugs that may protect against CCl_4 induced hepatotoxicity in rats. In these rats, urinary excretion of ascorbic acid is reduced. Measurement of urinary excretion of ascorbic acid thus serves as a parameter for hepatoprotective effect of a drug.

Hepatoprotective effects of other medicinal plants that have been evaluated in various models of liver diseases are given in Table 1.

12.3. Pancreato-protectives

The protective effect of *Emblica officinalis* in experimentally induced acute necrotising pancreatitis in dogs has been evaluated by Thorat *et al*,[148]. *Emblica officinalis* inhibited the increase in serum amylase caused by pancreatitis. Microscopical examination showed that the acinar cell damage and total inflammatory score was significantly less in dogs pretreated with *Emblica officinalis.*

12.4. Myelo-protectives

Withania somnifera prevented mylosuppression induced by one or more of the following 3 compounds, cyclophosphamide, azathioprine or prednisolone, as seen by a significant increase in haemoglobin concentration, RBC and WBC counts, platelet count and body weight and hemolytic antibody responses to human erythrocytes[149].

12.5. Radio-protectives

Oral administration of Rasayana group of drugs (from Ayurveda) were found to significantly increase total WBC count, bone marrow cellularity, natural killer cell and antibody dependent cellular cytotoxicity in gamma radiation exposed mice. Rasayanas reduced radiation induced lipid peroxidation in liver[150].

Methanolic extract (75%) of *Withania somnifera* (a plant belonging to the Rasayana group of drugs) significantly increased the WBC count in normal Balb/c mice and reduced the leucopenia induced by sublethal dose of gamma radiation. It also increased bone marrow cellularity and normalised the ratio of normochromatic to polychromatic erythrocytes following radiation. *Withania somnifera* probably exerts its effects by stimulating stem cell proliferation[151].

Active principles of *Withania somnifera* consisting of equimolar concentrations of sitoindosides VII-X and withaferin A induced a dose-related increase in superoxide dismutase, catalase and glutathione peroxide activities in rat brain frontal cortex and striatum, comparable to deprenyl, a known antioxidant[152].

Oral administration of anti-oxidants *viz.* curcumin, ellagic acid, bixin and alpha-tocopherol significantly decreased lung collagen hydroxyproline and thus lung fibrosis in rats following whole body irradiation. They also lowered serum and liver lipid peroxidation and liver superoxide dismutase activity and increased catalase activity. They also decreased the frequency of micronucleated polychromatic erythrocytes seen after whole body irradiation in mice[153].

The water extract of leaf of *Ocimum santum* was more effective and less toxic, as compared to aqueous ethanol extract, in improving the survival rate in mice, when administered intra-peritoneally before a whole body exposure to 11Gy of 60 Co gamma radiation. The intra-peritoneal route gave the best protection as compared to intra-muscular, intra-venous or oral routes[154].

12.6. Oculo-protectives

Leaves of *Ocimum sanctum* delayed the onset as well as the subsequent maturation of cataract significantly in 2 models of cataract *i.e.* galactosamic cataract in rats and naphthalene cataract in rabbits[155].

12.7. Membrane stabilizers

The leaf and root extracts of *Vernonia lasiopus* and *Vernonia galamensis* demonstrated prominent *in vitro* membrane stabilising property as determined by the percentage inhibition of RBC lysis [27].

13. Plants protecting against oxidative stress

13.1. UV light induced

Sobatum, purified from the plant *Solanum trilobatum* (Alarka) showed significant protection *in vitro* against UV light induced damage by free radicals on the bacteria *Salmonella typhimurium.* Similarly, it also protected against superoxide production that was generated by the reaction of photoreduced riboflavin and oxygen[156].

13.2. Cumene hydroperoxide induced

Tamra-bhasma, an organo-mineral compound from

Ayurveda, showed significant protection against cumene hydroperoxide induced lipid peroxidation and lowered reduced glutathione and superoxide dismutase levels in rat liver homogenate. It also significantly reduced malondealdehyde (MDA) levels. No alterations in biochemical and histopathological parameters was noted. The results thus suggested that tamra bhasma is a potent anti-oxidant drug and can be used in the management of lipid peroxidation[157].

Similarly, Sandhika, an Ayurvedic drug was evaluated *in vitro* using the same model (cumene hydroperoxide) and showed significant antioxidant activity[46].

13.3. Iron induced

Anti-peroxidative property of *Nardostachys jatamanasi* (Jatamanasi) was tested *in vitro* by using iron induced lipid peroxidation in rat liver homogenate. The degree of peroxidation was quantitated by thiobarbituric acid reactive substance (TBARS) content. Both the hexane and alcoholic extracts provided protection against lipid peroxidation (the hexane fraction was more potent) suggesting that the plant does have anti-oxidant activity[158].

Rubiadin, a dihydroxy anthraquinone, isolated from alcoholic extract of *Rubia cordifolia* (manjistha) demonstrated significant anti-oxidant property as it prevented lipid peroxidation induced by $FeSO_4$ and t-butylhydroperoxide (t-BHP) in a dose dependent manner. The percent inhibition was more in the case of Fe^{2+} induced lipid peroxidation. The anti-oxidant property of the preparation has been found to be better than that of EDTA, mannitol, Vitamin E and p-benzoquinone[159].

The potential of *Bacopa monniera* as an antioxidant was studied by Tripathi *et al*, [160]. The effect of the alcoholic and hexane fractions of *Bacopa monniera* on $FeSO_4$ and cumene hydroperoxide induced lipid peroxidation was studied. The alcoholic fraction showed greater protection against both that inducers and the results were comparable to known antioxidants like vitamin C. Probable mechanism of action could be through metal chelation at the initiation level and also as a chain breaker suggesting that *Bacopa monniera* is a potent anti-oxidant. The responses with *Bacopa monniera* were found to be dose-dependent. At low doses, it only slightly protected the auto-oxidation and $FeSO_4$ induced oxidation of reduced glutathione, but at higher concentrations, it enhanced the rate of oxidation.

14. Chemotherapeutic plant products

Plants which have shown anti-microbial, anti-fungal, anti-viral, anti-protozoal and anti-helminthic effects have been described in this section. Standard assays have been used by various investigators and most of the work has carried out *in vitro*.

14.1. Anti-microbial agents :

Clausenol, a carbazole alkaloid, isolated from an alcoholic extract of the stem bark of *Clausena anisata* was found to be active against gram positive and gram negative bacteria and fungi[161].

Substantial anti-microbial, anti-fungal and moderate insecticidal, sporicidal and cytotoxic activities were observed with the hexane extract of the stem bark of *Amona glabra*. Chromatographic fractionation of the stem led to the isolation of kaur-16-en-19-oic acid, which was found to be largely responsible for the biological activities observed[162].

The alcoholic extract of dry nuts of *Semecarpus anacardium* (Bhallatak) showed bactericidal activity *in vitro* against 3 gram negative strains (*Escherichia coli, Salmonella typhi and Proteus vulgaris*) and 2 gram positive strains (*Staphylococcus aureus and Corynebacterium diphtheriae*). Subsequent studies have shown that the alcoholic extracts of different parts of the plant (leaves, twigs, green fruit) also possess anti-bacterial properties, especially the leaf extract. No dermatoxic effect (irritant property) was observed in the mouse skin irritant assay[163].

No anti-bacterial activity with any extract of either the root or seeds of *Pongamia pinnata* was noted[3,4].

The acetone and alcoholic extracts of the leaves of *Cassia alata* showed significant *in vitro* anti-bacterial activity against *Staphylococcus aureus*, coagulase positive *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Bacillus stearothermophilus, Escherichia coli, Salmonella typhi and Salmonella. dysentriae.* In addition, the alcoholic extract also inhibited growth of *Klebsiella pneumoniae* whereas the acetone extract inhibited the growth of Vibrio cholerae[164].

Due to a lack of ideal diffusion and evaporation from the surface it is generally difficult to assess the anti-bacterial properties of aromatic oils derived from plants using the agar cup and disc diffusion methods. Hence, Agnihotri and Vaidya [165] have to developed a novel approach to study the anti-bacterial property of certain plants like *Eugenia caryophyllus, Thymus vulgaris, Cinnamonum zeylanium and Cuminum cyminum.* Volatile components of the hexane extracts of these plants were tested against standard gram positive and gram negative bacteria grown on agar slants and the results were expressed as percentage inhibition of the area of the slants. Of the 4 plants selected, *Thymus vulgaris* had the most prominent anti-bacterial activity.

14.2. Anti-fungal agents

The ethanolic extract of *Azadirachta indica* leaves demonstrated much more significant anti-dermatophytic activity as compared to the aqueous extract, when tested *in vitro* against 88 clinical isolates of dermatophytes using the agar dilution technique. The MIC90 of ethanolic extract was 100 µg/ml whereas that of aqueous extract was 500 µg/ml[166].

Four Siddha drugs *viz Nandhi mezhugh, Parangi pattai choornam, Erasa kenthi mezhugu* and *Vaan mezhugu* (in order of efficacy) were found to have significant anti-fungal activity when tested against 14 strains of *Candida albicans*[167].

Essential oil obtained from the herb of *Santolina chamaecyparissus* showed significant anti-fungal activity both *in vitro* (against 13 strains of *Candida albicans*) and *in vivo* (experimentally induced vaginal and systemic candidiasis in mice)[168]. It also showed activity against experimentally induced superficial cutaneous mycoses in guinea pigs by the hair root invasion test[169]. Anti-bacterial activity was also observed as seen by its inhibitory effects on the growth of *Staphylococcus aureus, Bacillus subtitis, Bacillus caerus* and *Escherichia coli.*

Rai[170] screened 17-medicinal plants against the test pathogen, *Pestalotiopsis mangiferae* and the results revealed that 14 plants had anti-mycotic activity whereas 3-plants, *viz., Argemone mexicana, Caesalpinia bonducella* and *Casia fistula* accelerated the growth of the pathogen. Maximum antimycotic activity was shown by *Eucalyptus globulus* (88%) and *Catharanthus roseus* (88%) followed by *Ocimum sanctum* (85.50%), *Azadirachta indica* (84.66%), *Ricinus communis* (Erand) (75%) and *Lawsonia inermis* (74.33%) while the minimum activity was exhibited by *Jatropha curcas* (10%).

The essential oil isolated from the leaves of *Aegle marmelos* exhibited significant anti-fungal activity against different fungal isolates and 100% inhibition of spore germination of all the tested fungi when evaluated using the spore germination assay. Kinetic studies showed that the inhibition was both concentration as well as time dependent[171].

Four compounds have been isolated from an extract prepared from the fruit rind of *Terminalia belerica viz.* termilignan, thannilignan (both lignans), 7-hydroxy-3',4'-(methylenedioxy) flavone and anolignan B. All possessed demonstrable anti-HIV-1, anti-malarial, and anti-fungal activity *in vitro* [172,173].

The natural xanthones isolated from the fruit hulls of *Garcinia mangostana* showed good inhibitory activity against the three phytopathogenic fungi, *Fusarium vasinfectum, Alternaria tenuis*, and *Dreschlera oryzae.* Substitution in the A and C rings in the derivatives of mangostin obtained by has been shown to modify the bioactivities of the compounds[174].

The petroleum ether, chloroform, acetone and ethanol (95%) extracts of the leaves of *Cassia alata* also showed significant *in vitro* anti-fungal activity against various fungi *viz. Aspergillus niger, R. japonicum, Candida albicans, C. tropiathis and R. glutinis*[175].

The root of *Withania somnifera* was found to be effective in prolonging the survival of Balb/c mice infected intravenously with *Apergillus fumigatus.* This protective activity is probably due to the observed increase in phagocytosis and intracellular killing capacity of peritoneal macrophages induced by treatment with *Withania somnifera*, thus suggesting that the plant has the potential to activate macrophage function in infectious states[176].

14.3. Anti-viral agents

Although initial studies by Thyagarajan *et al,* [177] with *Phyllanthus amarus* showed promising results in Hepatitis B carriers, further studies have demonstrated that the plant does not clear the hepatitis B surface antigen (HbsAg) in asymptomatic carriers of the antigen[178]. However, recently, in an *in vitro* study, the aqueous extract of *Phyllanthus amarus* was

Table 1. Illustrates the other plants that have been evaluated in various models of liver diseases and the parameters selected for their evaluation.

Plants/Plant derived agents (Author)	Damaging agent (Animals)	Parameters of evaluation
Methanolic extracts of the seeds of *Apium graveolens* Linn. and *Hygrophilia auriculata* H [268]	Paracetamol)) rats Thioacetamide)	Liver function tests (AST, ALT, alkaline phosphatase, sorbitol dehydrogenase, glutamate dehydrogenase and bilirubin), Hepatic triglycerides, Histopathology.
50% alcoholic extract of *Phyllanthus emblica* and its isolate, quercetin[269]	Country made liquor ingestion (rats). Paracetamol (mice)	Liver function tests, Histopathology.
Garlic oils[270]	Radiocalcium (mice)	Hepatic total lipids, triglycerides, phospholipids, free fatty acids
Swertia chirata[271]	CCl_4 (rats)	Liver function tests, Liver glycogen content, Serum cholesterol, Histopathology.
Ethyl acetate extract of *Acacia catechu* (katha) [272]	CCl_4 (rats)	Liver function tests, Histopathology.
Leaf extract of *Glycosmis pentaphylla*[273]	CCl_4 (rats)	Liver function tests, Histopathology.
Propolis, a natural resin produced by honey bees, rich in flavonoids[274]	Alcohol and CCl_4 (rats)	Liver function tests (AST, ALT), Blood and hepatic glutathione levels, Hepatic lipid peroxide content, Histopathology.
Powdered roots and aerial parts of *Sida rhombifolia* and their aque us extracts[41]	CCl_4, paracetamol and rifampicin (rats)	Liver function tests
Plant leaf suspension and methanolic extract of *Trichopus zeylanicus*[275]	Paracetamol (rats)	Liver function tests, Histopathology, Hepatic and lipid peroxide contents.
Methanolic extract of *Trichopus zeylanicus* [275]	Normal rats	Cholerectic activity

incubated with the Alexandar cell line, a human hepatocellular carcinoma derived cell line which has the property of secreting the Hepatitis B surface antigen (HbsAg) in the supernatant. The results demonstrated that *Phyllanthus amarus* was effective in inhibiting the secretion of HbsAg for 48 hrs thus proving its anti-hepatitis B virus property at the cellular level[179].

Glycyrrhizin, a triterpenoid glycoside obtained from *Glycyrrhiza glabra* (Yasthimadhu) was tested against RNA viruses like the Chandripura virus, Measles virus, Polio vaccine viruses type 1, 2 and 3, Polio wild type viruses 1, 2 and 3 as well as DNA viruses like the Herpes type 1 and 2 viruses *in vitro*. It inhibited the DNA virus plaque formation at lower concentrations (0.608 mM) while the RNA viruses were inhibited at higher concentrations (1.216 mM)[180].

Premanathan *et al*,[181] carried out *in vitro* screening of mangrove plant extracts for anti-immunodeficiency virus activity. HIV infected MT-4 cells were incubated with the extract and anti-viral activity was detected using tetrazolium-based colorimetric assay. Seven extracts were found to be effective five of which (bark of *Rhizophora mucronata* and leaves of *Excoecaria agallocha*, *Ceriops decandra*, *Rhizophora apiculata*, *Rhizophora lamarckii*) completely inhibited the virus adsorption to the cells.

14.4. Anti-protozoal agents

14.4.1. Antimalarial :

Ethanolic and petroleum extracts of *Artemisia japonica, Artemisia maritimia* and *Artemisia nilegarica* were tested for anti-malarial activity, both *in vivo* and *in vitro*. *In vivo* studies were carried out in Balb/c mice using the Rane test wherein all the compounds prolonged the survival time of the mice. *In vitro*, all 3 compounds inhibited schizont maturation in chloroquine sensitive strains of *Plasmodium falciparum*[182]

Ball shaped wood scrappings soaked in 5% Neem oil (*Azadirachta indica*) diluted in acetone and placed in water storage overhead tanks controlled the breeding of *Anopheles stephensi* and *Aedes aegypti* in 45 days[183]. Similarly, application of a cream of *Azadirachta indica* on exposed body parts at the rate of 2.0 gm/person significantly protected against Aedes, Culex and Anopheles musquitoe bites[184].

14.4.2. Anti-leishmanial :

The methanolic extract of *Swertia chirata* was found to inhibit the catalytic activity of topoisomerase I enzyme of *Leishmania donovani*. On subjecting the extract to fractionation, it yielded 3 secoiridoid glycosides, amarogentin, amaroswerin and sweroside of which amacogentin was found to be a potent inhibitor of topoisomerase I and exerted its effect by interacting with the enzyme, thus preventing binary complex formation[185].

14.4.3. Anti-trypanosomial

Crude 50% ethanolic extract of *Parthenium hysterophorus* flowers exhibited trypanocidal activity against *Trypanosoma evansi* both *in vitro* and *in vivo*. Toxicity was seen only at 1g / kg dose[186].

14.5. Anthelminthic agents

14.5.1. Anti-Nematodes

Kumar *et al* [187] has studied the mechanism of action of palasonin, the active principle of *Butea frondosa* seeds on *Ascaridia galli*. Palasonin inhibited glucose uptake and depleted the glycogen content[187] and thus the possible mechanism of its anthelminthic action may be related to inhibition of energy metabolism.

Both aqueous and alcoholic extracts of the leaves of *Sencio nudicaulis* Buch Ham were found to exert anti-filarial activity when tested against *Setaria cervi* (Nematoda Filarioidea). The effective concentrations differed for the aqueous and alcoholic extracts suggesting the presence of a cuticular permeability barrier. Both extracts also demonstrated micro-filaricidal action *in vitro*. Their anti-filarial responses were similar to diethylcarbamazine in that they too did not block the stimulant effect of acetylcholine on the worm[188].

Co-administration of Regulipid, a herbal formulation, with diethylcarbamazine therapy to patients suffering from filariasis was found to decrease chyluria in these patients[189].

Mustafa *et al*[190] injected the excretory-secretory products released by the adult *Setaria cervi*, a bovine filarial parasite, into rabbits to raise polyvalent antibodies. These antibodies can be used to detect circulating antigens in sera by counter immuno-electrophoresis and serve as a diagnostic test for filariasis.

14.5.2. Anti-Trematode (fluke)

The root tuber extract of *Flemingia vestita*, an indigenous medicinal plant in Meghalaya, exhibited anti-helminthic activity *in vitro*, against 2 species of flukes, *Artyfechinostomum sufrartyfex* and *Fasciolopsis buski*. It caused paralysis in both the species. Stereoscanning observations on the tegumental surfaces revealed sloughing off of most of the spines or their deformation and wrinkling and rupture of the general tegument[191].

14.5.3. Agents with molluscicidal activity

The leaf, bark, cake and oil of *Azadirachta indica* and synthetic pesticides derived from the plant demonstrated both, dose and time dependent, molluscicidal activity when tested against the snails, *Lymnaea* *acuminata and Indoplanorbis exustus.* The cidal effect of pure azadirachtin was greater then that of the synthetic molluscicides[192].

15. Anti-mutagenic plants

Punark, a mixture of solvent extracts of natural products, namely turmeric (*Curcuma longa*), betel leaf (*Piper betel*) and catechu (*Acacia catechu*) protected against benzo (a) pyrene induced chromosomal damage in human lymphocytes *in vitro* [193]. Alcoholic extracts of tumeric oil (TD) and tumeric oleoresin (TOR) showed anti-mutagenic effect *in vitro*. They also demonstrated chemoprotective effect in lymphocytes of normal healthy subjects *in vitro* when tested against benzo (a) pyrene induced DNA damage. *In vivo* the extracts reduced DNA damage (cytogenetic damage) in oral mucosal cells of patients with oral submucous fibrosis[194].

Water, oil and alcoholic extracts of nuts of *Semecarpus anacardium* were found to be anti-mutagenic when tested against benzo (a) pyrene (BZP) in the bacterial test system using *Salmonella typhimurium* strains TA98 and TA100. The water extract was less effective as compared to the oil and alcoholic extracts. In addition, the water and alcoholic extracts showed anti-mutaginic effect when tested in lymphocyte cultures of normal healthy volunteers[195].

Ellagic acid, a fraction isolated from *Terminalia arjuna* has been evaluated for it anti-mutagenic potential in TA98 and TA100 strains of *Salmonella typhimurium* against direct and indirect - acting mutagens. The fraction was quite effective against S9-dependent 2AF while it showed moderate effect against NPD[196].

16. Anti-cancer plants

The potential role of various plants in cancer therapy as either a direct anti-cancer agent, chemopreventive agent, radiosensitizer or immunity enhancer is presented in the following paragraphs.

Evaluation of the *in vitro* anti-cancer effects of bioflavonoids, *viz.* quercelon, catechin, luteolin and rutin against human carcinoma of larynx (Hep-2) and sarcoma 180 (S-180) cell lines showed that only luteolin and quercetin inhibited the proliferation of the cells. Luteolin caused depletion of glutathione in the cells and a decline in DNA synthesis, as seen by ^3H thymidine uptake studies, thus demonstrating its anticancer potential[197].

The anti-tumor effect of the crude extract of *Centella asiatica* as well as its partially purified fraction was studied in both, *in vitro* short and long term chemosensitivity test systems and *in vivo* tumor models. The purified fraction inhibited the proliferation of transformed cell lines of Ehrlich ascites tumor cells and Dalton's lymphoma ascites tumor cells more significantly than the crude extract. It also significantly suppressed the multiplication of mouse lung fibroblast cells in long term culture. *In vivo* administration of both extracts retarded the development of solid and ascites tumors and increased the lifespan of the tumor bearing mice. Tritiated thymidine, uridine and leucine incorporation assays suggest that the purified fraction acts directly on DNA synthesis[198].

The methanol eluted fraction of the petroleum ether extract of the root bark of *Salacia oblonga* Wall showed 100% cytotoxicity on Ehrlich ascites tumor cells[86].

Fresh root suspension of *Janakia arayalpathra* exhibited strong anti-tumor effects in mice challenged with Ehrlich Ascitic Carcinoma (EAC) cells. It prolonged the survival of all mice and protected a number of mice from tumor growth, probably by enhancing the activity of the immune system[199].

Withaferin A, a steroidal lactone isolated from the roots of *Withania somnifera*, reduced survival of V79 cells in a dose-dependent manner. The applicability of this drug as a radiosensitizer in cancer therapy needs to be explored[200].

Banerjee *et al*, [201] have studied the modulatory influence of the alcoholic extract of leaves of *Ocimum sanctum* on various enzyme levels in the liver, lung and stomach of mouse. Oral treatment with the extract significantly elevated the activities of cytochrome P450, cytochrome b5, arylhydrocarbon hydroxylase and glutathione S-transferase enzymes, all of which are important in the detoxification of carcinogens as well as mutagens. Moreover, it also significantly elevated extra-hepatic glutathione S-transferase and reduced glutathione levels in the liver, lung and stomach. These observations suggest that the leaf extract or its active principles may have a potential role in the chemoprevention of chemical carcinogenesis.

Studies conducted by Rao *et al,* [202] have shown that pergularinine (PgL) and tylophorinidine (TPD) isolated from *Pergularia pallida* are potently toxic and inhibit the growth of *Lactobacillus leichmannii* cells by binding to thymidylate synthetase. The binding led to significant inhibition of thymidylate synthetase activity making them potential anti-tumor agents.

Petroleum ether extract of *Hygrophilic spinosa* exhibited anti-tumor activity in Ehlrich ascitic carcinoma and sarcoma 180 bearing mice [203].

Aqueous extract of *Podophyllum hexandrum*, a herb from the Himalayas, demonstrated significant anti-tumor effects when drug was tested in strain 'A' mice carrying solid tumors developed by transplanting Ehrlich ascites tumor cells. Radioprotective effects were also seen when the drug was administrated to mice before whole body lethal irradiation of 10 Gy[204].

The chemopreventive efficacy of *Trianthema portulacastrum* L.Aizoaceae was tested in male Sprague-Dawley rats. Hepatocarcinogenesis was induced by the potent carcinogen diethylnitrosoamine (DENA). Treatment of the rats with aqueous, ethanolic and chloroform fractions of the plant extract at a dose of 100 mg/kg once daily reduced the incidence, numerical preponderance, multiplicity and size distribution of visible neoplastic nodules. Morphometric evaluation of focal lesions showed a reduction in number of altered liver cell foci per square centimeter as well as of average area of individual lesion. A decrease in the percentage of liver parenchyma occupied by foci seems to suggest the anti-carcinogenic potential of the plant extract in DENA-induced hepatocarcinogenesis[205].

Pretreatment with *Ocimum sanctum* leaf extract followed by the addition of 7,12-dimethylbenz[a]anthracene (DMBA) significantly blocked the formation of DMBA-DNA adducts in primary cultures of rat hepatocytes *in vitro.* The viability of the cells was not adversely affected by the extract[206].

17. Immune active plants

Modulation of the immune response through stimulation or suppression may help in maintaining a disease free state. Agents that activate host defence mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy. Upadhyay[207] has highlighted the therapeutic potential of immunomodulatory angets from plant products. They have evaluated Indian medicinal plants for immunomodulatory activity[208]. The authors have also reviewed the Ayurvedic concepts of preventive health care. A list of Ayurvedic medicinal plants showing immunomodulatory activity has been provided which includes agents like *Withania somnifera, Allium sativum, Azadirachta indica, Piper longum, Asparagus racemosus, Glycyrrhiza glabra, Aloe vera, Gmelina arborea* and *Tinospora cordifolia.*

Thatte and Dahanukar, [209] have described how clues from the description of ancient writings can lead to the development of new immunostimulatory agents. The experiments carried out to prove the rasayana concept of Ayurveda have demonstrated that *Asparagus racemosus, Tinospora cordifolia* and *Withania somnifera* protected animals against infections in normal and immunosuppressed states induced by hemisplenectomy or surgery[210]. These plants also produced leucocytosis with predominant neutrophilia and prevented, to varying degrees, the leucopenia induced by cyclophosphomide. They were found to activate the polymorphonuclear and monocyte-macrophage systems. Only those rasayanas which produced sweet (madhur) vipaka (*Tinospora cordifolia, Asparagus racemosus, Emblica officinalis, Terminalia chebula and Withania somnifera*) were found to stimulate the reticulo-endothelial system, but not those like *Acorus calamus, Commiphora mukul* and *Picorrhiza kurroa*, which produced bitter (katu) vipaka[210].

Among the immunostimulant rasayanas, *Tinospora cordifolia* has been extensively studied by Dahanukar *et al* [211]. It has been found to activate the mononuclear cells to release cytokines like GMCSF [212] and IL-1 in a dose dependent manner [210]. Whole aqueous extract of *Tinospora cordifolia*, standardized using HPTLC, has been evaluated as an adjuvant in clinical conditions like obstructive jaundice, tuberculosis and cancer chemotherapy and has been found to increase the efficacy of conventional therapy[210]. Active principles of *Tinospora cordifolia* were found to possess anticomplementary and immunomodulatory activities. Syringin (TC-4) and cordiol (TC-7) inhibited the *in vitro* immunohaemolysis of antibody - coated sheep erythrocytes by guinea pig serum by inhibiting the C3-convertase of the classical complement pathway. The compounds also gave rise to significant increases in IgG antibodies in serum. Both humoral and cell-mediated immunity were dose-dependently enhanced. Macrophage activation was reported for cordioside (TC-2), cordiofolioside A (TC-5) and cordiol (TC-7) and this activation was more pronounced with increasing incubation times[213].

The effect of *Asparagus racemosus, Tinospora cordifolia, Withania somnifera* and *Picorrhiza kurroa* on macrophage function obtained from mice treated with the carcinogen, ochratoxin (OTA) was evaluated by Dhuley[214]. Treatment with these plants significantly attenuated the OTA-induced suppression of chemotactic activity as well as IL-1 and TNF-α production by macrophages. Moreover, *Withania somnifera* potentiated macrophage chemotaxis and *Asparagus racemosus* induced excessive production of TNF-α as compared to controls.

Ray *et al*[215] demonstrated that ovalbumin immunized mice treated with *Azadirachta indica* leaf extract had higher IgG and IgM levels and anti-ovalbumin antibody titres as compared to control (humoral response). *Azadirachta indica* also induced cell mediated response as seen from the enhancement of macrophage migration inhibition and footpad thickness. These findings were supported by Ansari *et al*, [216]. They found that *Azadirachta indica* potentiated the antibody titres following typhoid H. antigen immunisation and induced delayed hypersensitivity following administration of tuberculin and DNCB to animals. In human volunteers, it stimulated humoral immunity by increasing antibody levels and cell mediated immunity by increasing total lymphocyte and T-cell count in 21 days.

Oral pretreatment with leaf extract of *Azadirachta indica* reversed the inhibitory effect of restraint stress on formation of anti-sheep RBC antibody titres in rats immunized with sheep RBC and also the increase in foot pad thickness. It reversed the DDT induced suppression of antibody response and leucocyte migration inhibition in tetanus toxoid immunized rats. Restraint stress along with administration of DDT in subthreshold doses resulted in an inhibition of the immune response. *Azadirachta indica* attenuated the immunotoxicity of environmental and xenobiotic stressors[217].

Root suspension of *Janakia arayalpathra* was found to have immunostimulatory properties in mice. It stimulated an increase in humoral antibody titres and also of antibody secreting spleen cells in the plaque forming cells assay following immunisation with sheep erythrocytes. It also increased the number of peritoneal macrophages and produced an increase in delayed hypersensitivity reaction in mice [199].

The alkaloidal fraction of *Boerrhiva diffusa* significantly restored the suppressed humoral response in stressed rats as observed by Mungantiwar *et al*,[218] wherein *Boerrhiva diffusa* increased the suppressed antibody titres following immunization by sheep RBCs in rats subjected to restraint stress. It also significantly reversed the depleted adrenal cortisol level and the elevated plasma cortisol level in the stressed rats, thus appearing to have a corticosteroid sparing effect in experimental stress.

Immune-21, a polyherbal natural product, has been shown to exhibit significant immunopotentiating and immuno-prophylactic activity, both *in vitro* and *in vivo*[219].

18. Adaptogens

Adaptogen is a term used to describe agents that increase the nonspecific resistance of organisms against a variety of stressors. A recent review on adaptogens, [211] describes the developments taking place in this field and the problems associated in the evaluation of adaptogens.

In a series of experiments the whole, aqueous standardised extracts of *Tinospora cordifolia, Asparagus racemosus, Emblica officinalis, Withania somnifera, Piper longum* and *Terminalia chebula*, were administered orally to experimental animals, in a dose extrapolated from the human dose. These animals were exposed to a variety of biological, physical and chemical stressors. The plants were found to offer protection against these stressors[211]. All the plants reversed the effects of cisplatin on gastric emptying, while *Tinospora cordifolia* and *Asparagus racemosus* also normalised cisplatin induced intestinal hyper-motility, complying to the definition of an adaptogen. They were found to be safe in both acute and subacute toxicity studies. All of them produced immunostimulation[211]. The type of extract (methanolic extract of *Withania somnifera* was more active) and time of administration (the best effects were observed only if given as pre-treatment) also influenced the effects. Of these plants *Emblica officinalis* strengthened the defense mechanisms against free radical damage induced during stress. The effect of *Emblica officinalis* appeared to depend on the ability of target tissues to synthesize prostaglandins. On the other hand, gastroprotective effect of *Tinospora cordifolia* was probably mediated through a predominantly immunostimulant mechanism as the protection was found to disappear on blocking the macrophage function.

In normal mice, high doses of *Tinospora cordifolia* significantly increased apoptosis in bone marrow cells. The therapeutic doses (100-200 mg/kg) were devoid of such effect. However, at the same therapeutic doses, it induced apoptosis in malignant cells, but protected the normal bone marrow from apoptosis induced by cyclophosphamide. This variable effect of Tinospora cordifolia (of increasing or decreasing apoptosis) depending on the stressor (either cancer or cyclophosphamide) as well as the cell type (S-180 or bone marrow cells) suggests its true potential as an adaptogen. It is of further interest to note that *Tinospora cordifolia* increases the bone marrow proliferative fractions at 100 and 200 mg/kg doses thus leading to leucocytosis. If the dose is increased, apoptosis is observed and the leucocytosis is blunted. This apparent paradox may be due to its effects on c-myc, a gene that causes both proliferation as well as induces apoptosis depending on the environment. It is exciting thus to hypothesise that *Tinospora cordifolia* may be producing some of its effects via activation of c-myc and inducing 'genotypic' adaptation.

Ocimum sanctum, known to have antistress properties, was recently studied by Sembulingam *et al*, [220] for its antistress effects against a different type of stress *i.e.* noise pollution, in rats. The ethanolic extract of *Ocimum sanctum* reversed the changes in plasma levels of corticosterone induced by exposure to both acute and chronic noise stress, indicating the antistress property of the plant against noise.

Studies have been reported in the literature to explore the possible mechanisms responsible for adaptogenic effect. For example, *Panax ginseng* did not modify brain and hypothalamic 5HT levels in unstressed rats, however, it attenuated restraint stress induced elevation of 5HT levels[221].

19. Nutraceutics

This is an emerging field of therapy. As we come to the end of this millennium, more and more people are getting health conscious and are looking at dietary substances for preventive or curative effects. Support towards this line of thinking from the scientific field is described in the following paragraphs.

19.1. Indian spinach (Beta vulgaris)

Dietary consumption of green vegetables has been associated with protection against mutagenic and clastogenic activity of genotoxicants. Chlorophyll, present in all green plants, has been suggested to be the principal factor involved. Sarkar *et al*, [222] compared the clastogenic or anticlastogenic effects of crude aqueous extract of leaf of Indian spinach, (*Beta vulgaris* L. var. benghalensis Hort.) and equivalent amounts of chlorophyll extracted from leaf, purified chlorophyll and chlorophyllin (a sodium copper derivative). After treatment for 7 days, the mice were administered potassium dichromate, a known metallic clastogen and sacrificed 24 hrs later. Cytogenetic end points were chromosomal aberrations and damaged cells. Results showed that both crude leaf extract and chlorophyllin were nonclastogenic and reduced the clastogenic effects of potassium dichromate. However, chlorophyll was clastogenic. The protective effect of the crude leaf extract was attributed to the total effect of the interaction between the different components within the leaf extract, thus neutralising the clastogenic effects of chlorophyll.

19.2. Karela (Momordia charantia)

Substitution of groundnut oil with palmolein in cereal based lactovegetarian diets provides about 30% of total fat calories, doubles the saturated fatty acids and reduces by half the linoleic acid content. The hypoglycemic activity of the alcoholic extract of the pulp of *Momordia charantia* (Karela) was evaluated in 3 experimental models of diabetes. In the normal glucose primed rat model, it decreased plasma glucose that was not accompanied by increase in insulin secretion. No evidence of tachyphylaxis to its effects on repeated dosing was found. In streptozotocin induced diabetic rats, it improved glucose tolerance and significantly reduced plasma glucose. The extract also increased the rate of glycogen synthesis from ^{14}C- glucose by 4-5 fold in the liver of normal rats. All the results suggest that the mechanism of action of *Momordia charantia* could be partly attributed to increased glucose utilisation in the liver rather than an insulin secretion effect[223].

19.3. Edible oils

Ghafoorunissa *et al*, [224] studied the effects of the substitution of palmolein oil for ground nut oil on selected cardiovascular risk factors and membrane functions in middle aged subjects. The effects were essentially similar with both treatment regimes. This study indicates that palm oil may not produce the deleterious effects associated with saturated fatty acids[225]. The author further explained that the tocols present in palm oil are natural biological anti-oxidants and can therefore augment the anti-oxidant potential of Indian diets. Also, red palm oil is the richest natural source of carotenes (especially beta-carotene) which are powerful biological anti-oxidants and hence, red palm oil can be used to prevent vitamin A deficiency which is widespread in India.

A study conducted by Kumar, [226] ruled out the association of increased incidence of coronary heart disease (CHD) with the high consumption of coconut and coconut oil in Kerala. Since their consumption of coconut and coconut oil and saturated fats and found that both groups did not differ in 32 CHD patients and 16 age and sex matched healthy controls.

19.4. Turmeric (Curcuma longa)

Curcumin (from *Curcuma longa*) protected against decrease in heart rate and blood pressure and biochemical changes in cat heart after coronary artery ligation. It also prevented the elevation of MDA content and lactate dehydrogenase release in the ischaemic zone. However, it did not prevent the increase in myeloperoxidase activity, indicating that curcumin protects against ischaemia induced changes by increasing the antioxidant defense mechanisms[227].

Deshpande *et al*, [228] demonstrated that both pre-treatment as well as concurrent treatment of turmeric extract in CCl_4 treated rats caused a reduction in cholesterol, bilirubin, SGOT, SGPT and alkaline phosphatase activity; concurrent treatment offering more significant protection.

The anti-mutagenic activity of Curcumin has already been described under the section 'Anti-mutagenic plants'.

19.5. Fenugreek (Trigonella foenum graecum)

Administration of unroasted and roasted powdered forms of seeds of *Trigonella foenum graecum* (fenugreek) to alloxan induced diabetic rats produced a significant fall in various serum lipids like total cholesterol, LDL and VLDL cholesterol and triglycerides in normal rats and in addition, increased HDL cholesterol in diabetic rats[229].

19.6. Curry leaf (Murraya Koenigii) and Mustard (Brassica juncea)

Whole curry leaf (*Murraya Koenigii*) and mustard (*Brassica juncea*) fed to rats at doses equal to normal human intake did not cause any adverse effect on food efficiency ratio, haematological parameters, liver and renal function tests, fibrin level and glycosylated haemoglobin. No histopathological changes were observed in the liver[230]. Both, plants showed significant hypoglycemic action in rats. There was an increase in the concentration of hepatic glycogen and glycogenesis and a decrease in glycogenolysis and gluconeogenesis [231].

The status of lipid peroxidation was investigated in rats fed *Murraya Koenigii* and *Brassica juncea*. Concentration of malondialdehyde showed a significant decrease, while hydroperoxides and conjugated dienes were significantly increased in liver and heart of both the experimental groups. Superoxide dismutase and catalase activity was found to be increased in liver and heart of both the spices administered groups. Glutathione levels in liver, heart and kidney were lowered in rats administered these species. Glutathione reductase, glutathione peroxidase and glutathione S-transferase activity showed a sharp increase in the experimental group compared to the controls[232].

19.7. Mint leaf (Mentha spicata)

Mint leaf has been shown to have significant stimulatory effect on the lipase activity of pancreas and intestinal mucosa in rats. It also stimulated intestinal amylase activity[233]. It, however, had no effect on bile secretion and its composition.

19.8. Onion (Allium cepa)

Augusti[234] in his review on the therapeutic values of onion (*Allium cepa*) and garlic (*Allium sativum*), has discussed the presence of many sulfur containing active principles mainly in the form of cysteine derivatives in onion and garlic, that are responsible for the various biological activities such as anti-diabetic, antibiotic, hypocholesterolaemic and fibrinolytic.

19.9. Garlic (Allium sativum)

S-allyl cysteine sulfoxide, isolated from garlic (*Allium sativum*), has been shown to be as active as gugulipid in controlling hypercholestermia, obesity and derangement of enzyme activities in cholesterol diet fed rats. The beneficial effects are partly due to its inhibitory effects on transaminases, alkaline phosphatase, lipogenic enzymes and HMG CoA reductase and partly due to stimulatory effects on plasma lecithin-cholesterol acyl transferase lipolytic enzymes and fecal excretion of sterols and bile acids[235]. Further studies by these authors[236] showed that the treatment also reversed the lipid peroxidation and decrease in reduced glutathione levels, superoxide dismutase and catalase activities in cholesterol fed rats.

Garlic oil stimulated lipase activity only in the intestinal mucosa and reduced pancreatic trypsin and chymotrypsin activities. Like mint, garlic also did not show any effect on bile secretion and composition[233].

Garlic protein diet or daily administration of garlic oil to 2% cholesterol fed rats controlled significantly the increases in sulphated glycosaminoglycans in their heart and aorta. However, hyaluronic acid level increased. UDPG dehydrogenase decreased and several degrading enzymes increased in the aorta on treatment. The effects of treatment were just the reverse in liver. The high percentage of cysteine in garlic protein and the reactive disulphide group in the oil may be responsible for their beneficial effects[237].

Both garlic protein (16% of diet) and garlic oil (100 mg/kg/day) exhibited significant lipid lowering effects in rats fed with cholesterol diet. The hypolipidemic action is primarily due to a decrease in hepatic cholesterogenesis in the treated rats. Even though garlic oil was found to be more effective, the garlic protein is more palatable and free from an obnoxious smell[238].

Administration of water soluble proteins of garlic to alcohol fed rats caused a significant increase in antiperoxide activity and decrease in activity of glutathione peroxidase and gluthathione S-transferase[239].

19.10. Ginger (Zingiber officinalis)

The acetone and 50% alcoholic extracts of *Zingiber officinalis* (ginger) exhibited significant anti-emetic activity with the acetone extract being more effective than the ethanolic extract against emesis induced by 3 mg/kg cisplatin in healthy mongrel dogs[240]. These findings suggest that ginger could be an effective and cheap anti-emetic adjunct to cancer chemotherapy.

19.11. Nutmeg (Myristica fragrans)

Myristica fragrans (nutmeg) seed extract administration to hypercholesterolemic rabbits reduced both total and LDL cholesterol, lowered the cholesterol/phospholipid ratio and elevated the decreased HDL-ratio significantly. This extract also prevented the accumulation of cholesterol, phospholipids and triglycerides in liver, heart and aorta and dissolved atheromatous plaques of aorta. Fecal excretion of cholesterol and phospholipid were significantly increased in these rabbits[241].

The ethanolic extract of *Myristica fragrans* demonstrated significant hypolipidaemic effects in experimentally induced hyperlipidaemia in rabbits. It lowered the lipoprotein lipid levels, total cholesterol, LDL cholesterol and triglycerides. HDL cholesterol was not significantly affected. Total cholesterol:HDL and LDL: HDL ratios were also significantly lowered. It lowered the level of total cholesterol in the heart and liver and demonstrated platelet antiaggregatory activity[242].

19.12. Piperine (Piper nigrum and Piper longum)

Piperine, [1-[5-[1,3-benzodioxol-5-yl]-1-oxo-2,4, pentadienyl] piperidine], a pungent alkaloid present in *Piper nigrum* Linn, and *Piper longum* Linn. has been shown to enhance the bioavailability of various structurally and therapeutically diverse drugs. Data obtained from intestinal everted sacs studies suggest that piperine is absorbed very fast across the intestinal barrier through the transcellular pathway. It may act as an apolar molecule and form apolar complex with drugs and solutes. It may modulate membrane dynamics due to its easy partitioning thus helping in efficient permeability across the barriers[243].

However, in another study[244], Trikatu, a combination of *Piper longum, Piper nigrum* and *Zingiber officinalis* was found to decrease the bioavailability of isoniazid in rabbits as measured by the alteration of peak plasma concentration (C_{max}) levels and area under curve (AUC).

19.13. Betel leaf (Piper betle)

The influence of two varieties of betel leaf (*Piper betle* Linn.) namely, the pungent Mysore and non-pungent Ambadi, was examined on digestive enzymes of pancreas and intestinal mucosa and on bile secretion in experimental rats. The results indicated that while these betel leaves do not influence bile secretion and composition, they have a significant stimulatory influence on pancreatic lipase activity. The Ambadi variety of betel leaf has a positive stimulatory influence on intestinal digestive enzymes, especially lipase, amylase and disaccharidases whereas a slight lowering in the activity of these intestinal enzymes was seen when Mysore variety of betel leaf was administered. The latter variety also had a negative effect on pancreatic amylase. Further, both the betel leaf varieties have shown decreasing influence on pancreatic trypsin and chymotrypsin activities[245].

The anti-mutagenic potential has already been described earlier in this chapter.

19.14. Mowrah (Madhuca latifolia)

Mowrah (*Madhuca latifolia*) seeds are used as animal feed as they yield 40-50% edible fat and the meal contains saponins besides protein and high level of carbohydrates. However, the seeds were found to be toxic when administered to young and adult rats at levels of 10 to 40% in diet. The animals showed marked inhibition of feed intake and loss of body weight resulting in mortalities. Histopathological examination revealed a gradation of damage from slight erosion of the tip of villi of intestinal mucous membrane to complete necrosis and destruction of it, with increasing amounts of mowrah seed meal in diets. The other significant change was a severe vacuolar degeneration of kidney tubular cells[246].

20. Phytochemistry

Following are some of the studies carried out to find out the active principle of plants.

20.1. Clausena anisata

Two new carbozole alkaloids, designated as clausenol and clausenine, were isolated from alcoholic extract of the stem bark of *Clausena anisata.* Their structures were established as 1-hydroxy-6-methoxy-3-methylcarbazole and 1,6-dimethoxy-3-methyl carbazole, respectively, from physical and chemical evidence and synthesis. Clausenol was found to be active against gram-positive and gram-negative bacteria and fungi[161].

20.2. Albizia lebbeck

Three main saponins named albiziasaponins A, B and C were isolated from the bark of *Albizia lebbeck* and their structures were established through spectral analyses. These may be responsible for the antiallergic properties of *Albizia lebbeck*[247].

20.3. Ocimum sanctum

Gas liquid chromatographic analysis of fixed oil of *Ocimum sanctum* revealed the presence of five fatty acids (stearic, palmitic, oleic, linoleic and linolenic acids) which in further studies, demonstrated significant anti-inflammatory activity[35,36].

20.4. Bacopa monniera

Chemical characterization of the plant, *Bacopa monniera* has been carried out by Garai *et al*[248,249]. Three new dammarane-type triterpenoid saponins, bacopasaponins A,B and C and a new dammarane type pseudojujubogenin glycoside, bacopa saponin D have been isolated and identified by spectroscopic methods and some chemical transformations.

20.5. Cerpegia juncea

From the chloroform extract of finely chopped, shade dried whole plant of *Cerpegia juncea* cerpegin has been derived which is the active principle responsible for its analgesic properties[30].

20.6. Ochna obtusata

Column chromatography of chloroform extract shade-dried and powdered stem bark of *Ochna obtusata* afforded an orange compound, crystallised from acetone which gave elemental analysis, IR, UV, HNMR and MS data corresponding to Prezewalskinone-B. This is probably the active principle that is responsible for the analgesic and anti-inflammatory properties of the plant[250].

20.7. Camellia sinensis

Air-dried roots of *Camellia sinensis* were extracted by percolation with methanol at ambient temperature. Solvent extraction, chromatography and hydrolysis procedures progressively yielded 3-0-β-D-glucopyranoso-a-spinasterol. Further studies will have to be carried out to correlate these principles with the pharmacological (hypoglycaemic and ulceroprotective) activities of the plant[251].

20.8. Phytolectins

Lectins are structurally diverse, carbohydrate binding proteins that bind reversibly to specific mono-or oligosaccharides. They are being used by the biomedical scientists and biochemists in blood typing and stimulation of cell for chromosome analysis and gene mapping, in cell separation, identification of complex glycoproteins and typing bacteria. Cell targeting by lectins in cancer therapy is still in its infancy. Sengupta *et al*, [252] have reviewed the potential of these biomolecules in medicine.

Lectin activities in roots, nodules, stems and leaves of 1-6 week old peanut plant (*A.hypogaea*) were checked by erythrocyte (human and rabbit) agglutination and sugar inhibition assays. Human and rabbit erythrocyte agglutinating activities were specifically inhibited by lactose/cellobiose (SLII) and methyl alpha-mannoside (SLI) respectively. Seed embryos and cotyledons agglutinated neuraminidase treated human erythrocytes and that activity was inhibited by T-disaccharide. In the roots of field grown plants SLI was the major activity, while nodules showed both activities (SLI and SLII). Specific activities of SLI and SLII were maximal in stem tissue and minimal hypocotyl. Actively growing tissues contained more SLII activity in comparison to the mature tissues. Immunological tests indicated that all the vegetative tissue lectins are serologically related[253].

21. Miscellaneous

21.1. Reviews on medicinal plants

It is beyond the scope of this chapter to justify review articles that have been published in the last 5 years. For the benefit of the readers, the articles are summarised in the following paragraphs. An interested reader may refer to them for detailed information.

Suresh, *et al*, [254] have investigated the phytochemical and pharmacological activities of 25 medicinal plants, commonly used by the tribals of Nilgiris, using various experimental models *viz.,* CNS-active plants (*Araucaria bidwilli, Brachylepsis nervosa*), plants with analgesic activity (*Araucaria bidwilli, Brynopsis lacinosa, Cyclea peltata, Ipomoea obscura, Mirabilis jalappa, Santolina chamaeccyparissus, Stephania japonica*), anti-inflammatory plants (*A. houstanianum, Araucana bidwilli, Bauhinia variegate, Iberis amara, Ipomoea obscura, Mirabilis jalappa, Santolina chamoeccyparissus,Stephania japonica, Thunbergia fragrans*), antipyretic plants (*Araucaria bidwilli, Malvastrum coromandelianum, Rumex nepalensis, Santolena chamaeccyparissus, Stephamia japonicum, Toddalia asiatica*), plants with local anaesthetic activity (*Mirabilis jalappa*), plants affecting smooth muscle (relaxant effect) (*Araucaria bidwilli, Bauhinia variegata, Brachylepsis nervosa, Calotropis gigantea, Cardiospermum helicacabum, Impomea obscura, Malvastrum coromandelianum, Melianthus major, Rubia cordifolia, Stephania japonica, Thunbergia fragrans*), chemotherapeutic agents (Brachylepsis nervosa, Calotropis gigantea, Ipomoea obscura), plants modulating fertility (*Ailanthus excelsea*), CVS active plants (*Cystisus scoparius, Cystisus scoparius*), diuretic plants (*Cystisus scoparius, Sida cordifolia, Toddalia asiatica*), ulceroprotectives (*Araucaria bidwilli, Malvastrum coromandelianum, Santolina chamaeccyparissus*), anti-diarrhoeal plants (*Bauhinia variegata, Ipomoea obscura, Malothria perpusilla, Thunbergia fragrans*), haemostatic plants (*A houstanianum, Toddalia asiatica*) and effect on biochemical parameters (*Amarantus spinosus*).

Bhandary, *et al*, [255] conducted ethnomedical field study on 98 medicinal preparations, involving 69 species of plants, used by the Siddis of Uttara Kannada in the state of Karnataka. Their findings include 40 hitherto unknown medicinal uses of known medicinal plants. Among these, the use of the stem sap of *Calamus thwaitesii* as an antifertility drug, and the use of the flowers of *Ichnocarpus frutescens* and the rhizome of *Hedychium coronarium* in the treatment of diabetes are noteworthy. Aswal *et al*, [256] have described the results of their scientific endeavours in which the alcoholic extracts of 266 botanically identified plant materials from 222 plant species were tested for various biological activities including chemotherapeutic and pharmacological. Eighty-nine extracts were shown to possess biological activity. Follow-up studies have been carried out on some of these plants with confirmed activity. The active principles and results of these studies have also been discussed. The chemistry, pharmacology and traditional medicinal uses of various Vernonia species, also called as sahadevi, (*viz. Vernonia cinerea, Vernonia anthehelmintica, Vernonia amygdalina, Vernonia lasopius* etc.) have been reviewed by Johri and Singh[257]. Pharmacoepidemiological survey carried out by Karandikar *et al*,[1] in adults over 60 years of age revealed that about 47% of the elderly population uses herbal drugs. The main reason for herbal drug usage is the belief that these drugs have lesser side effects.

Nazarine, *et al*, [258] have screened two hundred and sixty extracts from marine organisms collected from the Western & Eastern coasts of India, Lakshadweep and the Andaman and Nicobar Islands, for their effects on 3 isolated tissues of the guinea pig namely, the ileum, the uterus and the atrium with the aim of detecting any anti-spasmodic, oxytocic. uterine relaxant, ionotropic and antiarrhythmic activity. Antispasmodic activity was observed in 22 extracts, ecbolic activity (spontaneous contractions in isolated uterus) in 59 samples, uterine relaxant activity in 16 samples and antihistaminic and anti-5HT activity in 6 samples.

21.2. Toxicity studies

Toxicity studies[16] revealed that LD50 of the ethanol extract of *Vitex leucoxylon* leaf was more than 3 g/kg whereas that of the cold aqueous infusion was 1050 mg/kg. LD_{50} of the ethanolic extracts was found to be 1 gm/kg for *Ailanthus excelsa*, 350 mg/kg for *Toddalia asiatica* and 250 mg/kg for *Araucaria bidwilli* on oral administration, in rats[49].

Cerpegin, a furopyridine alkaloid isolated from the chloroform extract of *Ceropegia juncea* was found to be toxic at doses above 400 mg/kg and the mice showed excitation, irritability, convulsions and respiratory paralysis[30].

The effect of multiple doses of the petroleum ether extract of *Hygrophilia spinosa* on the haematological and biochemical parameters and hepatorenal functions of normal mice was evaluated[259]. The results showed that weekly moderate to high dose levels (above 40 mg/kg) and daily high dose (8 mg/kg) affected liver and kidney functions and metabolic and haematological parameters. Lower doses did not alter them.

Sharathchandra and Balakrishnamurthy[260] have studied the mode of action of *Cleistanthus collinus*, a toxic plant that is frequently implicated in poisoning. *Cleistanthus collinus* causes a depletion of thiol/thiol containing enzymes in most organs which results in its toxicity. Thiol compounds may act as antidotes.

Administration of the juice of *Lantana camara* leaves to rats resulted in a significant reduction in the total protein, globulin, absolute lymphocyte count and percent lymphocyte count. Significant increase in the relative weights of adrenals was also observed. High doses (1500 mg/kg) significantly inhibited granulomatous tissue formation in rats, similar to cyclophosphamide[96].

The polyherbal drug, Prostina, recommended for use in benign prostatic hypertrophy showed no toxic effects as seen on morphological, gross behaviour, body weight changes and histopathological, biochemical and haematological changes in rats upto doses of 450 mg/kg, which is 15 times higher than the recommended dose[261].

22. Elemental analysis of plants drugs/formulations

Various Indian medicinal plants *viz. Ocimum sanctum, Tinospora cordifolia, Azadirachta indica, Nerium andicum* (Kanher) and *Acorus calamus* (Vacha) were analysed for the presence of minor and trace elements by instrumental neutron activation analysis (INAA). Concentrations of 13 elements were determined. *Zinc,* manganese, and sodium were significantly higher in *Ocimum sanctum* leaves while *zinc* was higher in *Azadirachta indica* leaves. The therapeutic significance of these plants in restoring ionic balance has been discussed by the authors[262].

In a similar study, specific parts of several plants (fruits, leaves, stem, bark and roots) often used as medicines in the Indian Ayurvedic system have been analysed by Singh and Garg[263] for 20 elements (As, Ba, Br, Ca, Cl, Co, Cr, Cu, Fe, K, Mn, Mo, Na, P, Rb, Sb, Sc, Se, Sr and Zn) by employing INAA. The samples were irradiated with thermal neutrons in a nuclear reactor and the induced activity was counted using high resolution gamma ray spectrometry. Most of the medicinal herbs have been found to be rich in one or more of the elements under study. Similarly, elemental analysis of some herbal plants used in the control of diabetes has been done by the techniques of Neutron Activation Analysis (NAA) and Atomic Absorption Spectroscopy (AAS). The elements Mn, Na, Cl, Al, Cu, Pb, Ni, Cr, Cd, Fe, Ca, Zn and Hg were found to be present in different plants in various proportions[264].

23. Modern correlates for standardisation

An attempt has been made by Uchil *et al* [265] to develop a battery of standardization tests using modern technology for commonly used organometallic preparations (bhasmas) and compare them with the Ayurvedic tests. Seven preparations of copper bhasma manufactured by different companies were screened using both the methods. The results revealed that only 3 preparations could fulfil at least 3 of 4 criteria as described in Ayurvedic textbooks. However, all of them differ with respect to particle size, bulk densities, copper content and presence of chemical groups and complexes when tested using modern correlates. None of them showed presence of elemental copper. These data reveal the necessity of development of modern correlates for standardisation of Ayurvedic formulation to add precision to quality control, to detect false claims and adulteration and to predict their adverse drug reactions.

24. Conclusion

We see a definite change in the pattern of research on medicinal plants. Our findings are listed below :

There is a growing interest in correlating phytochemical constituents of a plant with its pharmacological activity[266,267]. Scientists have even started correlating the botanical properties of plants with their pharmacological activity as seen from the work on by Rawat *et al* [146]. In future, more co-ordinated multidimensional research aimed at correlating botanical and phytochemical properties to specific pharmacological activities is expected.

In terms of pharmacological activity, more attention has been paid to CNS-active, cytoprotective, immunomodulators and chemotherapeutic plant products. Nutraceutics have opened up an entirely new field for exploration and, in the near future, dietary modulation of diseases may emerge as an alternative mode of therapy. At the same time a decreasing trend has been noticed towards evaluation of plants for their effects on the autonomic nervous system or fertility control.

With the advances in cellular biology, a shift towards studying changes in cytosolic enzyme activities, DNA patterns and genetic control has been observed rather than concentrating merely on the gross effects induced by the plant drugs,

In addition to the proper utilization of technological advances, a logical interpretation of the codified language of traditional medicine also becomes a necessity in order to further promote research in this field. The work done on rasayana group of plants[211] is a good example of the above statement. Here, the authors have attempted to interprete the word 'rasayana' in modern scientific terminology and have taken into consideration the advocated uses for this group of plants as per Ayurvedic textbooks while designing their research protocol. This understanding triggered the subsequent research work that was aimed at evaluating the immunostimulant potential of the rasayana group of plants and now, we have indigenous immunostimulants available, at affordable rates, in the Indian market.

However, there is a flip side too:

Very few articles published in the last 5 years have provided adequate information on the procedures adapted by the researchers for quality assurance of the plant products. Any publication related to phytopharmacology should ideally provide data on the authentication and standardisation of the plant products.

Not all the research areas selected by the scientists were relevant to needs of our country. Infections like tuberculosis, malaria, diarrhoea, AIDS and malnutrition are some of the major problems of our country. However, these areas have not been extensively explored.

Majority of the drugs are at the experimental stage and have to still undergo clinical trials. In 1982, Dr.Satyavati had expressed need for well planned clinical studies. 16 years later, the status has not changed. There is still a paucity of clinical studies which are carried out in randomized, controlled, double blind manner.

Today, concurrent consumption of drugs from different disciplies is a common finding. Very few studies, however, address the problem of drug interactions, if any. In view of this, two studies documenting interaction of plant drugs with modern medicine are important from the clinician's point of view[26,58]. Similarly, the documented data on adverse drug reactions of plant drugs is also sparse.

A relevant point that arose while compiling this data and needs mention is the need for documentation of research activities and publications of results, whether positive or negative, in peer-reviewed journals. Although, work carried out on traditional medicine is presented in conferences and is available in the conference abstract books (data of which may or may not be complete) or published in local journals, these are not peer-reviewed and moreover this data is accessible to a select few. This results in lacunae while judging the current status of research on traditional medicine and leads to a false impression that not enough research is being carried out in this field. Hence, networking of the various research activities carried out by different scientists has now become the need of the hour in order to provide information about and access to research work done in different laboratories of the country.

REFERENCES

1. Karandikar SM, Pandit VA, Kulkarni SD. Pharmaco-epidemiological study of use of herbal medicines in the elderly in Pune. *Bharati Vidyapeeth Bulletin* 1997;**1**:9-11.
2. Vaidya AB. The status and scope of Indian medicinal plants acting on central nervous system. *Indian J Pharmacol* 1997;**29**:S340-3.
3. Singh RK, Joshi VK, Goel RK, *et al.* Pharmacological actions of *Pongamia pinnata* seeds - a preliminary study. *Indian J Exp Biol* 1996;**34**:1204-7.
4. Singh RK, Nath G, Acharya SB, *et al.* Pharmacological actions of *Pongamia pinnata* roots in albino rats. *Indian J Exp Biol* 1997;**35**:831-6.
5. Kumar A, Singh RK. Effect of *Pongamia pinnata* on perturbed central cholinergic markers of cognition in experimentally demented rats. *J Basic Appl Biomed* 1996;**4**:43-8.
6. Singh HK, Dhawan BN. Neuropsychopharmacological effects of the ayurvedic nootropic *Bacopa monniera* Linn. *(Brahmi) Indian J Pharmacol* 1997;**29**:S359-65.
7. Iyer MR, Pal SC, Kasture VS, Kasture SB. Effect of *Lawsonia inermis* on memory and behaviour mediated *via* monoamine neurotransmitters. *Indian J Pharmacol* 1998;**30**:181-5.
8. Bhattacharya SK. Nootropic effect of BR-16A (Mentat), a psychotropic herbal formulation, on cognitive deficits induced by prenatal undernutrition, postnatal environmental impoverishment and hypoxia in rats. *Indian J Exp Biol* 1994;**32**:31-6.
9. Faruqi S, Andrade C, Ramteke S, *et al.* Herbal pharmacotherapy for the attenuation of electroconvulsive shock-induced anterograde and retrograde amnestic deficits. *Convuls Ther* 1995;**11**:241-7.
10. Handa SS, Bhargava VK. Effect of BR-16A (MentatR) on cognitive deficits in aluminium-treated and aged rats. *Indian J Pharmacol* 1997;**29**:258-61.
11. Ramteke S, Andrade C, Faruqi S, *et al.* BR-16A attenuates anterograde amnesia induced by electro-convulsive shocks in slow-learning rats. *Indian J Pharmacol* 1995; **27**:186-8.
12. Bhardwaj SK, Srivastava KK. Effect of a composite Indian herbal preparation, CIHP(III) on avoidance learning during endurance performance of rats. *Indian J Exp Biol* 1995;**33**: 580-4.
13. Bhattacharya SK, Kumar A. Effect of Trasina, an Ayurvedic herbal formulation, on experimental models of Alzheimer's disease and central cholinergic markers in rats. *J Altern Complement Med* 1997;**3**:327-36.
14. Vinekar AS, Andrade C, Sriprada VT, *et al.* Attenuation of ECS-induced retrograde amnesia by using an herbal formulation. *J ECT* 1998;**14**:83-8.
15. Jaiswal AK, Bhattacharya SK, Acharya SB. Anxiolytic activity of *Azadirachta indica* leaf extract in rats. *Indian J Exp Biol* 1994;**32**:489-91.
16. Makwana HG, Ravishankar B, Shukla VJ, *et al.* General pharmacology of *Vitex leucoxylon* Linn leaves. *Indian J Physiol Pharmacol* 1994;**38**:95-100.
17. Mukherjee PK, Saha K, Balasubramanian R, *et al.* Studies on pyschopharmacological effects of *Nelumbo nucifera* Gaertn. rhizome extract. *J Ethnopharmacol* 1996;**54**:63-7.
18. Mitra SK, Chakraborti A, Bhattacharya SK. Neuropharmacological studies on *Panax ginseng. Indian J Exp Biol* 1996;**34**:41-7.
19. Singh RK, Nath G, Goel RK, Bhattacharya SK. Pharmacological actions of Abies pindrow Royle leaf. *Indian J Expt Biol* 1998;**36**:187-91.
20. Satyan KS, Jaiswal AK, Ghosal S, Bhattacharya SK. Anxiolytic activity of ginkgolic acid conjugates from Indian Ginkgo biloba. *Psychopharmacology (Berl)* 1998;**136**:148-52.
21. Bhattacharya SK. Behavioural studies on BR-16A (Mentat), a herbal psychotropic formulation. *Indian J Exp Biol* 1994; **32**:37-43.
22. Kulkarni SK, Ninan I. Inhibition of morphine tolerance and

dependence by *Withania somnifera* in mice. *J Ethnopharmacol* 1997;**57**:213-7.

23. Manocha A, Pillai KK, Husain Z. Influence of *Ginkgo biloba* on the effect of anticonvulsants. *Indian J Pharmacol* 1996; **28**:84-7.

24. Manocha A, Pillai KK, Husain Z. Effect of *Ginkgo biloba* in chemoshock in mice. *Indian J Pharmacol* 1997;**29**:198-200.

25. Kulkarni SK, George B. Pentylenetetrazol-induced kindling in animals: protective effect of BR-16A. *Indian J Exp Biol* 1995;**33**;424-7.

26. Vaz J, Kulkarni C, David J, Joseph T. Influence of caffeine on pharmacokinetic profile of sodium valproate and carbamazepine in normal human volunteers. *Indian J Exp Biol* 1998;**36**:112-4.

27. Johri RK, Singh C, Kaul BL. *Vernonia lasiopus* and *Vernonia galamensis*: A medicinal perspective. *Res Ind* 1995; **40**:327-8.

28. Ramaswamy S, Viswanathan S. Influence of gossypin on the development of acute tolerance to morphine induced antinociception. *Indian J Expt Biol* 1997;**35**:413-4.

29. Khanna N, Goswami M, Sen P, Ray A. Antinociceptive action of *Azadirachta indica* (neem) in mice: possible mechanisms involved. *Indian J Exp Biol* 1995;**33**:848-50.

30. Sukumar E, Hamsaveni GR, Bhima RR. Pharmacological actions of cerpegin, a novel pyridine alkaloid from *Ceropegia juncea*. *Fitoterapia* 1995;**66**:403-6.

31. Sivaprakasam P, Viswanathan S, Thirugnanasambantham P, Reddy MK, Vijayasekaran V. Pharmacological screening of *Ochna obtusata*. *Fitoterapia* 1996;**67**:117-20.

32. Narayanan N, Thirugnanasambantham P, Viswanathan S, *et al.* Antinociceptive antiinflammatory and antipyretic effects of ethanol extract of *Clerodendron serratum* roots in experimental animals. *J Ethnopharmacol* 1998 (accepted for publication).

33. Kumar VL, Basu N. Anti-inflammatory activity of the latex of *Calotropis procera*. *J Ethnopharmacol* 1994;**44**:123-5.

34. Mengi SA, Deshpande SG. Evaluation of ocular anti-inflammatory activity of *Butea frondosa*. *Indian J Pharmacol* 1995;**27**:116-9.

35. Singh S, Majumdar DK, Yadav MR. Chemical and pharmacological studies on fixed oil of *Ocimum sanctum*. *Indian J Exp Biol* 1996;**34**:1212-5.

36. Singh S, Majumdar DK. Evaluation of antiinflammatory activity of fatty acids of *Ocimum sanctum* fixed oil. *Indian J Exp Biol* 1997;**35**:380-3.

37. Singh RK, Pandey BL. Anti-inflammatory potential of *Pongamia pinnata* root extracts in experimentally induced inflammation in rats. *J Basic Appl Biomed* 1996;4:21-4.

38. Singh RK, Pandey BL. Anti-inflammatory activity of seed extracts of *Pongamia pinnata* in rat. *Indian J Physiol Pharmacol* 1996;**40**:355-8.

39. Singh RK, Pandey BL. Further study of anti-inflammatory effects of *Abies pindrow*. *Phytother Res* 1997;**11**:535-7.

40. Vimala R, Nagarajan S, Alam M, Susan T, Joy S. Anti-inflammatory and antipyretic activity of *Michelia champaca* Linn, (white variety), *Ixora brachiata Roxb.* and *Rhynchosia cana* (Willd.) D.C. flower extract. *Indian J Exp Biol* 1997;**35**:1310-14.

41. Rao KS, Mishra SH. Anti-inflammatory and hepatoprotective activities of *Sida rhombifolia* Linn. *Indian J Pharmacol* 1997;**29**:110-6.

42. Ismail TS, Gopalakrishnan S, Hazeena BV. Biochemical modes of action of *Gmelina asiatica* in inflammation. *Indian J Pharmacol* 1997;**29**:306-9.

43. Mukherjee PK, Saha K, Pal M, Saha BP. Studies on the anti-inflammatory activity of rhizomes of *Nelumbo nucifera* (letter). *Planta Med* 1997;**63**:367-9.

44. Chattopadhyay RR. Possible biochemical mode of anti-inflammatory action of *Azadirachta indica* A. Juss in rats. *Indian J Exp Biol* 1998;**36**:418-20.

45. Diwan PV, Margaret I, Ramakrishna S. Influence of *Gymnema sylvestre* I on inflammation. *Inflammapharmacol* 1995;3:271-77.

46. Chaurasia S, Tripathi P, Tripathi YB. Antioxidant and anti-inflammatory property of Sandhika: a compound herbal drug. *Indian J Exp Biol* 1995;**33**:428-32.

47. Chatterjee S, Das SN. Anti-arthritic and anti-inflammatory effect of a poly-herbal drug (Ease(R)) : Its mechanism of action. *Indian J Pharmacol* 1996;**28**:116-9.

48. Karunakar N, Pillai KK, Husain SZ, Rao M. Investigations of anti-inflammatory activity of Jigrine. *Indian J Physiol Pharmacol* 1997;**41**:134-8.

49. Suresh B, Dhanasekaran S, Elango K, *et al.* Anti-pyretic activity of some plants in female albino rats : A preliminary report. *Ancient Sci Life* 1995;**14**:253-7.

50. Subramoniam A, Pushpangadan P, Rajasekharan S, Latha PG. Antipyretic activity of TBR-002, A herbal formulation. *Ancient Sci Life* 1995;**15**:7-14.

51. Mukherjee PK, Das J, Saha K, *et al.* Antipyretic activity of *Nelumbo nucifera* rhizome extract. *Indian J Exp Biol* 1996;**34**:275-6.

52. Ganguli S, Hota D, Goel RK, *et al.* Neuropharmacological studies on Fusarium toxins-III : Neurochemical investigations on total toxin extracts from *F. moniliforme* and *F. oxysporum*. *Indian J Exp Biol* 1996;**34**:531-4.

53. Andrade C, Raj T, Udaya HB, Suresh Chandra J. Effect of

BR-16A on alpha-2 adrenergic, dopamine autoreceptor and dopamine postsynaptic receptor functioning. *Indian J Pharmacol* 1994;**26**:292-5.

54. Kayath HP, Goel NK. Effects of cuscuta stem extract on various animal tissues. *Indian J Pharmacol* 1995;**27**:227-9.

55. Das M, Vedasiromoni JR, Chauhan SP, Ganguly DK. Effect of green tea (*Camellia sinensis*) extract on the rat diaphragm. *J Ethnopharmacol* 1997;**57**:197-201.

56. Bhattacharya P, Bhatt JD, Hemavathi KG, Gulati OD. Effects of chronic terbutaline and Abana pretreatments on beta-adrenoceptor mediated relaxant responses of guinea-pig isolated trachea. *Indian J Pharmacol* 1994;**26**:270-6.

57. Dhanasekaran S, Ravishankar R, Sumitra Devi K, *et al.* Pharmacological studies of *Araucaria Bidwillii* hook. *Geobios New Reports* 1994l;**13**:49-52.

58. Dalvi SS, Nayak VK, Pohujani SM, *et al.* Effect of gugulipid on bioavailability of diltiazem and propranolol. *J Assoc Phys India* 1994;**42**:454-5.

59. Srinivasan MR, Srinivasan K. Hypocholesterolemic efficacy of garlic-smelling flower *Adenocalymma alliaceum* Miers. in experimental rats. *Indian J Exp Biol* 1995;**33**:64-6.

60. Sharma A, Mathur R, Dixit VP. Hypocholesterolemic activity of nut shell extract of *Semecarpus anacardium* (Bhilawa) in cholesterol fed rabbits. *Indian J Exp Biol* 1995;**33**:444-8.

61. Shaila HP, Udupa AL, Udupa SL. Preventive actions of *Terminalia belerica* in experimentally induced atherosclerosis. *Int J Cardiol* 1995;**49**:101-6.

62. Ram A. Effect of *Plumbago zeylanica* in hyperlipidaemic rabbits and its modification by vitamin E. *Indian J Pharmacol* 1996;**28**:161-6.

63. Dwivedi S. Effect of *Plumbago zeylanica* in hyperlipidemic rabbits and its modification by vitamin E. (Letter to the Editor). *Indian J Pharmacol* 1997;**29**:138.

64. Bopanna KN, Bhagyalakshmi N, Rathod SP, *et al.* Cell culture derived *Hemidesmus indicus* in the prevention of hypercholesterolemia in normal and hyperlipidemic rats. *Indian J Pharmacol* 1997;**29**:105-9.

65. Srividya N, Periwal S. Diuretic, hypotensive and hypoglycaemic effect of *Phyllanthus amarus. Indian J Exp Biol* 1995;**33**:861-4.

66. Chattopadhyay RR. Effect of *Azadirachta indica* hydro-alcoholic leaf extract on the cardiovascular system. *Gen Pharmacol* 1997;**28**:449-51.

67. Bhatt JD, Panchakshari UD, Hemavati KG, Gulati OD. Effect of Abana, an Ayurvedic preparation of ethinyl estradiol-induced hypertension in rats. *Indian J Pharmacol* 1998;**30**; 399-403.

68. Nyman U, Joshi P, Madsen LB, *et al.* Ethnomedical information and *in vitro* screening for angiotensin-converting enzyme inhibition of plants utilized as traditional medicines in Gujarat, Rajasthan and Kerala (India). *J Ethnopharmacol* 1998;**60**:247-63.

69. Chopra K, Singh M. Involvement of oxygen free radicals in cardioprotective effect of rutin-A naturally occurring flavonoid. *Indian J Pharmacol* 1994;**26**:13-18.

70. Vaidya AB. *Terminalia chebula* in cardiovascular therapy. *J Assoc Phys India* 1994;**42**:281-2.

71. Bharani A, Ganguly A, Bhargava KD. Salutary effect of *Terminalia arjuna* in patients with severe refractory heart failure. *Int J Cardiol* 1995;**49**:191-9.

72. Dwivedi S, Jauhari R. Beneficial effects of *Terminalia arjuna* in coronary artery disease. *Indian Heart J* 1997;**49**:507-10.

73. Mukherjee PK, Saha K, Bhattacharya S, *et al.* Studies on antitussive activity of *Drymaria cordata* Willd. *J Ethnopharmacol* 1997;**56**:77-80.

74. Saha K, Mukherjee PK, Murugesan T, *et al.* Studies on *in vivo* antitussive activity of *Leucas lavandulaefolia* using a cough model induced by sulfur dioxide gas in mice. *J Ethnopharmacol* 1997;**57**:89-92.

75. Govindan S, Viswanathan S, Vijayasekaran V, Alagappan R. A pilot study on the clinical efficacy of *Solanum xanthocarpum* and *Solanum trilobatum* in bronchial asthma. *J Ethnopharmacol* 1998 (accepted for publication).

76. Nair AM, Tamhankar CP, Saraf MN. Studies on the mast cell stabilising activity of *Vitex negundo* Linn. *Indian Drugs* 1994;**32**:277-82.

77. Nair AM, Saraf MN. Inhibition of antigen and compound 48/80 induced contractions of guinea pig trachea by the ethanolic extract of the leaves of *Vitex negundo* Linn. *Indian J Pharmacol* 1995;**27**:230-3.

78. Gupta PP, Srimal RC, Srivastava M, *et al.* Antiallergic activity of arbortristosides from *Nyctanthus arbortristis. Int J Pharmacognosy* 1995;**33**:70-2.

79. Gupta PP, Tandon JS, Patnaik GK. Antiallergic activity of andrographolides isolated from *Andrographis paniculata* (Burm.F) Wall. *Int J Pharmacognosy* 1997;**36**:1-3.

80. Gupta PP, Kulshrestha DK, Patnaik GK. Antiallergic activity of *Cedrus deodara. J Med Aromatic Plant Sci* 1997;**19**: 1007-8.

81. Barua CC, Gupta PP, Patnaik GK, *et al.* Studies on antianaphylactic activity of fractions of *Albizzia lebbeck. Cur Sci* 1997;**72**:397-9.

82. Rizvi SI, Abu ZM, Suhail M. Insulin-mimetic effect of (-) epicatechin on osmotic fragility of human erythrocytes. *Indian J Exp Biol* 1995;**33**:791-2.

83. Manickam M, Ramanathan M, Jahromi MA, *et al.* Antihyperglycemic activity of phenolics from *Pterocarpus marsupium. J Nat Prod* 1997;**60**:609-10.

84. Flexible dose open trial of Vijayasar in cases of newly-diagnosed non-insulin-dependent diabetes mellitus. Indian Council of Medical Research (ICMR), Collaborating Centres, New Delhi. *Indian J Med Res* 1998;**108**:24-9.

85. Augusti KT, Joseph P, Babu TD. Biologically active principles isolated from *Salacia oblonga* Wall. *Indian J Physiol Pharmacol* 1995;**39**:415-7.

86. Tripathi YB, Chaturvedi P. Assessment of endocrine response to *Inula racemosa* in relation to glucose homeostasis in rats. *Indian J Exp Biol* 1995;**33**:686-9.

87. Gomes A, Vedasiromoni JR, Das M, *et al.* Anti-hyperglycemic effect of black tea (*Camellia sinensis*) in rat. *J Ethnopharmacol* 1995;**45**:223-6.

88. Chattopadhyay RR. Possible mechanism of antihyperglycemic effect of *Azadirachta indica* leaf extract. Part IV. *Gen Pharmacol* 1996;**27**:431-4.

89. Seema PV, Sudha B, Padayatti PS, *et al.* Kinetic studies of purified malate dehydrogenase in liver of strepozotocin-diabetic rats and the effect of leaf extract of *Aegle marmelose* (L.) Correa ex Roxb. *Indian J Exp Biol* 1996; **34**:600-2.

90. Das AV, Padayatti PS, Paulose CS. Effect of leaf extract of *Aegle marmelose* (L.) Correa ex Roxb. on histological and ultrastructural changes in tissues of streptozotocin induced diabetic rats. *Indian J Exp Biol* 1996;**34**:341-5.

91. Subramoniam A, Pushpangadan P, Rajasekharan S, *et al.* Effects of *Artemisia pallens* Wall. on blood glucose levels in normal and alloxan-induced diabetic rats. *J Ethnopharmacol* 1996;**50**:13-7.

92. Saxena AM, Murthy PS, Mukherjee SK. Mode of action of three structurally different hypoglycemic agents: a comparative study. *Indian J Exp Biol* 1996;**34**:351-5.

93. Agrawal P, Rai V, Singh RB. Randomized placebo-controlled, single blind trial of holy basil leaves in patients with noninsulin-dependent diabetes mellitus. *Int J Clin Pharmacol Ther* 1996;**34**:406-9.

94. Rai V, Iyer U, Mani UV. Effect of Tulasi (*Ocimum sanctum*) leaf powder supplementation on blood sugar levels, serum lipids and tissue lipids in diabetic rats. *Plant Foods Hum Nutr* 1997;**50**:9-16.

95. Teotia S, Singh M. Hypoglycemic effect of *Prunus amygdalus* seeds in albino rabbits. *Indian J Exp Biol* 1997;**35**:295-6.

96. Garg SK, Shah MA, Garg KM, *et al.* Antilymphocytic and immunosuppressive effects of *Lantana camara* leaves in rats. *Indian J Exp Biol* 1997;**35**:1315-8.

97. Yadav P, Sarkar S, Bhatnagar D. Action of *Capparis decidua* against alloxan-induced oxidative stress and diabetes in rat tissues. *Pharmacol Res* 1997;**36**:221-8.

98. Dubey GP, Dixit SP, Singh A. Alloxan-induced diabetes in rabbits and effect of a herbal formulation D-400. *Indian J Pharmacol* 1994;**26**:225-6.

99. Dhawan D, Bandhu HK, Singh B, *et al.* Effect of D-400 (a herbal formulation) on the regulation of glucose metabolism in diabetic rats. *Indian J Pharmacol* 1996;**28**:224-6.

100. Bhattacharya SK, Satyan KS, Chakrabarti A. Effect of Trasina, an Ayurvedic herbal formulation, on pancreatic islet superoxide dismutase activity in hyperglycaemic rats. *Indian J Exp Biol* 1997;**35**:297-9.

101. Sheel J, Dehadrai S, Prakash AO. Estrogenic activity in the ethanolic extract of *Bupleurum marginatum. Indian J Pharmacol* 1995;**27**:258-61.

102. Talwar GP, Rahul Pal, Singh OM, *et al.* Safety of intrauterine administration of purified neem seed oil (*Praneem vilci*) in women and effect of its co-administration with the heterospecies driver birth control vaccine on antibody response to human chorionic gonadotropin. *Indian J Med Res* 1995;**102**:66-?.

103. Joshi AR, Ahamed RN, Pathan KM, Manivannan B. Effect of *Azadirachta indica* leaves on testis and its recovery in albino rats. *Indian J Exp Biol* 1996;**34**:1091-4.

104. Subramoniam A, Madhavachandran V, Rajasekharan S, Pushpangadan P. Aphrodisiac property of *Trichopus zeylanicus* extract in male mice. *J Ethnopharmacol* 1997;**57**:21-7.

105. Murthy DR, Reddy CM, Patil SB. Effect of benzene extract of *Hibiscus rosa* sinensis on the estrous cycle and ovarian activity in albino mice. *Biol Pharm Bull* 1997;**20**:756-8.

106. Deka DK, Lahon LC, Saikia J, Mukit A. Effect of *Cissus quadrangularis* in accelerating healing process of experimentally fractured radius-ulna of dog : a preliminary study. *Indian J Pharmacol* 1994;**26**:44-5.

107. Sarkar SN. Capillary permeability-increasing effect of *Eucalyptus hybrid* leaf and *Seseli indicum* seed oils in rabbit. *Indian J Pharmacol* 1994;**26**:55-6.

108. Rasik AM, Shukla A, Patnaik GK, *et al.* Wound healing activity of latex of *Euphorbia neriifolia* Linn. *Indian J Pharmacol* 1996;**28**:107-9.

109. Rao VG, Shivakumar HG, Parthasarathi G. Influence of aqueous extract of *Centella asiatica* (Brahmi) on experimental wounds in albino rats. *Indian J Pharmacol* 1996; **28**:249-53.

110. Suguna L, Sivakumar P, Chandrakasan G. Effects of *Centella asiatica* extract on dermal wound healing in rats. *Indian J Exp Biol* 1996;**34**:1208-11.

111. Sunilkumar, Parameshwaraiah S, Shivakumar HG. Evaluation of topical formulations of aqueous extract of *Centella asiatica* on open wounds in rats. *Indian J Exp Biol* 1998;**36**: 569-72.

112. Udupa SL, Udupa AL, Kulkarni DR. A comparative study on the effect of some indigenous drugs on normal and steroid-depressed healing. *Fitoterapia* 1998;**69**:507-10.

113. Paranjpe P, Kulkarni PH. Comparative efficacy of four Ayurvedic formulations in the treatment of acne vulgaris : a double-blind randomized placebo-controlled clinical evaluation. *J Ethnopharmacol* 1995;**49**:127-32.

114. Prasad KV, Bharathi K, Srinivasan KK. Evaluation of *Ammannia baccifera* Linn. for antiurolithic activity in albino rats. *Indian J Exp Biol* 1994;**32**:311-3.

115. Anand R, Patnaik GK, Roy K, Bhaduri AP. Antioxaluric and anticalciuric activity of lupeol derivatives. *Indian J Pharmacol* 1995;**27**:265-8.

116. Mukherjee PK, Das J, Balasubramanian R, *et al.* Antidiarrhoeal evaluation of *Nelumbo nucifera* rhizome extract. *Indian J Pharmacol* 1995;**27**:262-4.

117. Dahanukar SA, Rege NN, Thatte UM. Omnipotent officinale. *Amruth* 1998;**2**:21-3.

118. Maity S, Vedasiromoni JR, Ganguly DK. Anti-ulcer effect of the hot water extract of black tea (*Camellia sinensis*). *J Ethnopharmacol* 1995;**46**:167-74.

119. De B, Maiti RN, Joshi VK, Agrawal VK, Goel RK. Effect of some Sitavirya drugs on gastric secretion and ulceration. *Indian J Exp Biol* 1997;**35**:1084-7.

120. Goel RK, Maiti RN, Manickam M, Ray AB. Antiulcer activity of naturally occurring pyrano-coumarin and isocoumarins and their effect on prostanoid synthesis using human colonic mucosa. *Indian J Exp Biol* 1997;**35**:1080-3.

121. Manonmani S, Vishwanathan VP, Subramanian S, Govindawamy S. Biochemical studies on the antiulcerogenic activity of Cauvery 100, an ayurvedic formulation in experimental ulcers. *Indian J Pharmacol* 1995;**27**:101-5.

122. Mitra SK, Gopumadhavan S, Hemavathi TS, *et al.* Protective effect of UL-409, a herbal formulation against physical and chemical factor induced gastric and duodenal ulcers in experimental animals. *J Ethnopharmacol* 1996;**52**:165-9.

123. Vanisree AJ, Mitra K, Shyamala Devi CS. Antiulcerogenic effect of UL-409 against experimentally induced gastric ulcer in rats. *Indian J Pharmacol* 1996;**28**:265-8.

124. Kulkarni SK, Goel RK. Gastric antiulcer activity of UL-409 in rats. *Indian J Exp Biol* 1996;**34**:683-8.

125. Gupta I, Parihar A, Malhotra P, *et al.* Effects of *Boswellia serrata* gum resin in patients with ulcerative colitis. *Eur J Med Res* 1997;**2**:37-43.

126. Vaidya AB, Sirsat SM, Doshi JC, Antarkar DS. Selected medicinal plants and formulations as Hepato-biliary drugs - An overview. *Indian J Clin Pharmacol Ther* 1996;7-11.

127. Bhatt AD and Bhatt NS. Indigenous drugs and liver disease. *Indian J Gastroenterology* 1996;**15**:63-67.

128. Nagarkatti DS, Rege NN, Desai NK, Dahanukar SA. Modulation of Kupffer cell activity by *Tinospora cordifolia* in liver damage. *J Postgrad Med* 1994;**40**:65-7.

129. Bapat RD, Rege NN, Koti RS, Dahanukar SA. Can we do away with PTBD? HPB *Surgery* 1995;**9**:5-11.

130. Rege NN, Javle H, Bapat RD, *et al.* Antiendotoxic effect of *Tinospora cordifolia* : An experimental study in rats. *Indian J Surg* 1998;**60**:303-5.

131. Srivastava S, Srivastava AK, Srivastava S, Patnaik GK, Dhawan BN. Effect of picroliv and silymarin on liver regeneration in rats. *Indian J Pharmacol* 1994;**26**:19-22.

132. Rastogi R, Saksena S, Garg NK, *et al.* Picroliv protects against alcohol-induced chronic hepatotoxicity in rats. *Planta Med* 1996;**62**:283-5.

133. Visen PKS, Saraswat B, Patnaik GK, Agarwal DP, Dhawan BN. Protective activity of picroliv isolated from *Picrorhiza kurroa* against ethanol toxicity in isolated rat hepatocytes. *Indian J Pharmacol* 1996;**28**:98-101.

134. Saraswat B, Visen PK, Patnaik GK, Dhawan BN. Protective effect of picroliv, active constituent of *Picrorhiza kurroa,* against oxytetracycline induced hepatic damage. *Indian J Exp Biol* 1997;**35**:1302-5.

135. Ramesh C, Khanna AK, Dhawan BN. Picroliv regulates lipid metabolism in *Plasmodium berghei* induced liver damage in *Mastomys caucha. Indian J Pharmacol* 1997;**29(1)**: 39.

136. Santra A, Das S, Maity A, *et al.* Prevention of carbon tetrachloride-induced hepatic injury in mice by *Picrorhiza kurroa. Indian J Gastroenterol* 1998;**17**:6-9.

137. Koul IB, Kapil A. Effect of diterpenes from *Andrographis paniculata* on antioxidant defense system and lipid peroxidation. *Indian J Pharmacol* 1994;**26**:296-300.

138. Trivedi N, Rawal UM. Effect of aqueous extract of *Andrographis paniculata* on liver tumor. *Indian J Pharmacol* 1998;**30**:318-22.

139. Chauhan BL, Mohan AR, Kulkarni RD, Mitra SK. Bioassay for evaluation of the hepatoprotective effect of Liv.52, a polyherbal formulation, on ethanol metabolism in chronic alcohol-exposed rats. *Indian J Pharmacol* 1994;**26**:117-20.

140. Pandey S, Gujrati VR, Shanker K, Singh N, Dhawan KN. Hepatoprotective effect of Liv-52 against CCl_4 induced lipid

peroxidation in liver of rats. *Indian J Exp Biol* 1994; **32**:674-5.

141. Kataria M, Singh LN. Hepatoprotective effect of Liv-52 and kumaryasava on carbon tetrachloride induced hepatic damage in rats. *Indian J Exp Biol* 1997;**35**:655-7.

142. Suja V, Sharmila SL, Shyamala Devi C. Protective effect of Liv.52 and Liv.100, Ayurvedic formulations on lipid peroxidation in rat liver homogenate - an *in vitro* study. *Indian J Exp Biol* 1997;**35**:50-2.

143. Saraswathy SD, Suja V, Gurumurthy P, Shyamala Devi CS. Effect of Liv.100 against antitubercular drugs (isoniazid, rifampicin and pyrazinamide) induced hepatotoxicity in rats. *Indian J Pharmacol* 1998;**30**:233-8.

144. Kapur V, Pillai KK, Hussain SZ, Balani DK. Hepatoprotective activity of "Jigrine" on liver damage caused by alcohol-carbontetrachloride and paracetamol in rats. *Indian J Pharmacol* 1994;**26**;35-40.

145. Karunakar N, Pillai KK, Hussain SZ, *et al.* Further studies on the antihepatotoxic activity of Jigrine. *Indian J Pharmacol* 1997;**29**:222-7.

146. Rawat AK, Mehrotra S, Tripathi SC, Shome U. Hepatoprotective activity of *Boerhavia diffusa* L. roots - a popular Indian ethnomedicine. *J Ethnopharmacol* 1997;**56**:61-6.

147. Visweswaram D, Rajeswara Rao P, Satyanarayana S. A non-invasive method for screening hepatoprotective drugs against carbon tetrachloride induced hepatotoxicity. *Indian J Pharmacol* 1994;**26**:301-3.

148. Thorat SP, Rege NN, Naik AS, *et al. Emblica officinalis*: a novel therapy for acute pancreatitis - an experimental study. *HPB Surg* 1995;**9**:25-30.

149. Ziauddin M, Phansalkar N, Patki P, Diwanay S, Patwardhan B. Studies on the immunomodulatory effects of Ashwagandha. *J Ethnopharmacol* 1996;**50**:69-76.

150. Kumar P, Kuttan R, Kuttan G. Radioprotective effects of Rasayanas. *Indian J Exp Biol* 1996;**34**:848-50.

151. Kuttan G. Use of *Withania somnifera* Dunal as an adjuvant during radiation therapy. *Indian J Exp Biol* 1996;**34**:854-6.

152. Bhattacharya SK, Satyan KS, Ghosal S. Anxiolytic activity of glycowithanolides from *Withania somnifera. Indian J Exp Biol* 1997;**35**:236-9.

153. Thresiamma KC, George J, Kuttan R. Protective effect of curcumin, ellagic acid and bixin on radiation induced toxicity. *Indian J Exp Biol* 1996;**34**:845-7.

154. Devi PU, Ganasoundari A. Radioprotective effect of leaf extract of Indian medicinal plant *Ocimum sanctum. Indian J Exp Biol* 1995;**33**:205-8.

155. Sharma P, Kulshreshtha DK, Sharma AL. Anti-cataract activity of *Ocimum sanctum* on experimental cataract. *Indian J Pharmacol* 1998;**30**:16-20.

156. Mohanan PV, Rathinam K, Devi KS. Effect of Sobatum on ultra violet-induced damage and superoxide production. *Indian J Pharmacol* 1997;**29**:129-31.

157. Tripathi YB, Singh VP. Role of Tamra bhasma, an ayurvedic preparation, in the management of lipid peroxidation in liver of albino rats. *Indian J Exp Biol* 1996;**34**:66-70.

158. Tripathi YB, Tripathi E, Upadhyay A. Antilipid peroxidative property of *Nardostachys jatamanasi. Indian J Exp Biol* 1996;**34**:1150-1.

159. Tripathi YB, Sharma M, Manickam M. Rubiadin, a new antioxidant from *Rubia cordifolia. Indian J Biochem Biophys* 1997;**34**:302-6.

160. Tripathi YB, Chaurasia S, Tripathi E, Upadhyay A, Dubey GP. *Bacopa monniera* Linn. as an antioxidant: mechanism of action. *Indian J Exp Biol* 1996;**34**:523-6.

161. Chakraborty A, Chowdhury BK, Bhattacharya P. Clausenol and clausenine-two carbazole alkaloids from *Clausena anisata. Phytochemistry* 1995;**40**:295-8.

162. Padmaja V, Thankamany V, Hara N, *et al.* Biological activities of *Amona glabra. J Ethnopharmacol* 1995;**48**:21-24.

163. Nair A, Bhide SV. Antimicrobial properties of different parts of *Semecarpus anacardium. Indian Drugs* 1996;**33**:323-8.

164. Sakharkar PR and Patil AT. Antimicrobial activity of *Cassia alata. Indian J Pharmaceutical Sciences* 1998;**60**: 311-12.

165. Agnihotri S, Vaidya AD. A novel approach to study antibacterial properties of volatile components of selected Indian medicinal herbs. *Indian J Exp Biol* 1996;**34**:712-5.

166. Venugopal PV, Venugopal TV. Antidermatophytic activity of neem (*Azadirachta indica*) leaves *in vitro. Indian J Pharmacol* 1994;**26**:141-3.

167. Suresh B, Kalyanaraman VR, Dhanasekaran S, *et al.* Evaluation of certain Siddha drugs in the treatment of candidiasis. *Ancient Science of Life* 1994;**14**:16-20.

168. Suresh B, Sriram S, Dhanaraj SA, Elango K, Chinnaswamy K. Anticandidal activity of *Santolina chamaecyparissus* volatile oil. *J Ethnopharmacol* 1997;**55**:151-9.

169. Suresh B, Kalyanaraman VR, Dhanasekaran S, *et al.* Evaluation of Santolina oil in search of new drugs against candidiasis. *Indian J Pharmacol* 1995;**27**:171-7.

170. Rai MK. *In vitro* evaluation of medicinal plant extracts against *Pestalotiopsis mangiferae. Hindustan Antibiot Bull* 1996;**38**:53-6.

171. Rana BK, Singh UP, Taneja V. Antifungal activity of kinetics of inhibition by essential oil isolated from leaves of *Aegle marmelos. J Ethnopharmacol* 1997;**57**:29-34.

172. Valsaraj R, Pushpangandan P, Smitt UW *et al.* New anti-HIV-1, antimalarial, and antifungal compounds from *Terminalia belerica. J Nat Prod* 1997;**60**:739-42.

173. Valsaraj R, Pushpangadan P, Smitt UW,*et al.* Antimicrobial screening of selected medicinal plants from India. *J Ethnopharmacol* 1997;**58**:75-83.

174. Gopalakrishnan G, Banumathi B, Suresh G. Evaluation of the antifungal activity of natural xanthones from *Garcinia mangostana* and their synthetic derivatives. *J Nat Prod* 1997;**60**:519-24.

175. Sakharkar PR and Patil AT. Antifungal activity of *Cassia alata. Hamdard Medicus* 1998;**41**:20-1.

176. Dhuley JN. Therapeutic efficacy of Ashwagandha against experimental aspergillosis in mice. *Immunopharmacol Immunotoxicol* 1998;**20**:191-8.

177. Thyagaran SP, Subramanian S, Thirunalasunder, Venkateswaran PS, Blumberg BS. Effects of *Phyllanthus amarus* on chronic carriers of hepatitis B virus. *Lancet* 1988;**2**:764-6.

178. Doshi JC, Vaidya AB, Antarkar DS, *et al.* A two-stage clinical trial of *Phyllanthus amarus* in Hepatitis B carriers : Failure to eradicate the surface antigen. *Indian J Gastroenterol* 1994;**13**:7-8.

179. Jayaram S, Thyagarajan SP. Inhibition of HBsAg secretion from Alexander cell line by *Phyllanthus amarus. Indian J Pathol Microbiol* 1996;**39**:211-5.

180. Badam L. *In vitro* studies on the effect of glycyrrhizin from Indian *Glycyrrhiza glabra* Linn. on some RNA and DNA viruses. *Indian J Pharmacol* 1994;**26**:194-99.

181. Premanathan M, Nakashima H, Kathiresan K, *et al. In vitro* anti-human immunodeficiency virus activity of mangrove plants. *Indian J Med Res* 1996;**103**:278-81.

182. Valecha N, Biaswas S, Badoni V, *et al.* Antimalarial activity of *Artemisia japonica*, *Artemisia maritima* and *Artemisia nilegarica. Indian J Pharmacol* 1994;**26**;144-6.

183. Nagpal BN, Srivastava A, Sharma VP. Control of mosquito breeding using wood scrapings treated with neem oil. *Indian J Malariol* 1995;**32**:64-9.

184. Dua VK, Nagpal BN, Sharma VP. Repellent action of neem cream against mosquitoes. *Indian J Malariol* 1995;**32**:47-53.

185. Ray S, Majumder HK, Chakravarty AK, *et al.* Amarogentin, a naturally occurring secoiridoid glycoside and a newly recognized inhibitor of topoisomerase I from *Leishmania donovani. J Nat Prod* 1996;**59**:27-9.

186. Talakal TS, Dwivedi SK, Sharma SR. *In vitro* and *in vivo* therapeutic activity of *Parthenium hysterophorus* against *Trypanosoma evansi. Indian J Exp Biol* 1995;**33**:894-6.

187. Kumar D, Mishra SK, Tandan SK, Tripathi HC. Possible mechanism of anthelmintic action of palasonin on *Ascaridia galli. Indian J Pharmacol* 1995;**27**:161-8.

188. Singh R, Khan NU, Singhall KC. *In vitro* antifilarial activity of *Sencio nudicaulis* Buch. Ham. effect on *Setaria cervi* (*Nematoda Filarioidea*). *Indian J Physiol Pharmacol* 1996;**40**:231-6.

189. Samal KK, Panda RS, Parija BL, *et al.* Herbal preparation in treatment of chyluria of filarial origin. *J Assoc Phys India* 1995;**43**:876.

190. Mustafa H, Srivastava N, Kaushal DC, Kaushal NA. Analysis and potential of excretory-secretory antigens of *Setaria cervi* for immunodiagnosis of human filariasis. *Indian J Exp Biol* 1996;**34**:508-12.

191. Roy B, Tandon V. Effect of root-tuber extract of *Flemingia vestita*, a leguminous plant, on *Artyfechinostomum sufrartyfex* and *Fasciolopsis buski*: a scanning electron microscopy study. *Parasitol Res* 1996;**82**:248-52.

192. Singh K, Singh A, Singh DK. Molluscicidal activity of neem (*Azadirachta indica* A. Juss). *J Ethnopharmacol* 1996;**52**: 35-40.

193. Ghaisas SD, Bhide SV. *In vitro* studies on chemoprotective effect of Purnark against Benzo(A)pyrene-induced chromosomal damage in human lymphocytes. *Cell Biol (Int)* 1994;**18**:21-7.

194. Hastak K, Lubri N, Jakhi SD, *et al.* Effect of turmeric oil and turmeric oleoresin on cytogenetic damage in patients suffering from oral submucous fibrosis. *Cancer Letters* 1997;**116**:265-9.

195. Kothari AB, Lahiri M, Ghaisas SD, Bhide SV. *In vitro* studies on anti-mutagenecity of water, alcoholic and oil extracts of *Semecarpus anacardium. Indian J Pharmacol* 1997;**29**: 301-5.

196. Kaur S, Grover IS, Kumar S. Antimutagenic potential of ellagic acid isolated from *Terminalia arjuna. Indian J Exp Biol* 1997;**35**:478-82.

197. Elangovan V, Ramamoorthy N, Balasubramanian S, *et al.* Studies on the antiproliferative effect of some naturally occurring bioflavonoidal compounds against human carcinoma of larynx and sarcoma-180 cell lines. *Indian J Pharmacol* 1994;**26**:266-9.

198. Babu TD, Kuttan G, Padikkala J. Cytotoxic and anti-tumor properties of certain taxa of Umbelliferae with special reference to *Centella asiatica* (L.) Urban. *J Ethnopharmacol* 1995;**48**:53-7.

199. Subramoniam A, Rajasekharan S, Latha PG, *et al.* Immunomodulatory and antitumour activities of *Janakia arayalpathra* root. *Fitoterapia* 1996;**67**:140-4.

200. Devi PU, Akagi K, Ostapenko V, *et al.* Withaferin A: a new

radiosensitizer from the Indian medicinal plant *Withania somnifera*. *Int J Radiat Biol* 1996;**69**:193-7.

201. Banerjee S, Prashar R, Kumar A, Rao AR. Modulatory influence of alcoholic extract of Ocimum leaves on carcinogen-metabolizing enzyme activities and reduced glutathione levels in mouse. *Nutr Cancer* 1996;**25**:205-17.

202. Rao KN, Bhattacharya RK, Venkatachalam SR. Inhibition of thymidylate synthase and cell growth by the phenanthroindolizidine alkaloids pergularinine and tylophorinidine. *Chem Biol Interact* 1997;**106**:201-12.

203. Mazumdar UK, Gupta M, Maiti S, Mukherjee D. Antitumor activity of *Hygrophila spinosa* on Ehrlich ascites carcinoma and sarcoma-180 induced mice. *Indian J Exp Biol* 1997;**35**: 473-7.

204. Goel HC, Prasad J, Sharma A, Singh B. Antitumour and radioprotective action of *Podophyllum hexandrum*. *Indian J Exp Biol* 1998;**36**:583-7.

205. Bhattacharya S, Chatterjee M. Protective role of *Trianthema portulacastrum* against diethylnitrosoamine-induced experimental hepatocarcinogenesis. *Cancer Lett* 1998;**129**:7-13.

206. Prashar R, Kumar A, Hewer A, *et al.* Inhibition by an extract of *Ocimum sanctum* of DNA-binding activity of 7,12-dimethylbenz[a]anthracene in rat hepatocytes *in vitro*. *Cancer Lett* 1998;**128**:155-60.

207. Upadhyay SN. Therapeutic potential of Immunomodulatory agents from plant products. In: SN Upadhyay Ed. Immunomodulation, New Delhi, Narosa Publishing House, 1997;149-54.

208. Katiyar CK, Brindavanam NB, Tiwari P, Narayana DBA. Immunomodulator products from Ayurveda : current status and future prospectives. In: SN Upadhyay Ed. Immunomodulation, Narosa Publishing House, New Delhi 1997;163-87.

209. Thatte UM, Dahanukar SA. Rasayana Concept : Clues from immunomodulatory therapy. In: SN Upadhyay Ed. Immunomodulation, Narosa Publishing House, New Delhi 1997;141-8.

210. Dahanukar SA, Thatte UM. Current status of Ayurveda in Phytomedicine. *Phytomedicine* 1997;**4**:359-68.

211. Dahanukar SA, Rege NN, Thatte UM. Adaptogens. In: Ed. Medicinal plnats, their bioactivity, screening and evaluation - Proceedings of the International Workshop, CDRI, Lucknow (India), December 2-5, 1997;143-63.

212. Thatte UM, Rao SG, Dahanukar SA. *Tinospora cordifolia* induces colony stimulating activity in serum. *J Postgrad Med* 1994;**40**:202-3.

213. Kapil A, Sharma S. Immunopotentiating compounds from *Tinospora cordifolia*. *J Ethnopharmacol* 1997;**58**:89-95.

214. Dhuley JN. Effect of some Indian herbs on macrophage functions in ochratoxin A treated mice. *J Ethnopharmacol* 1997;**58**:15-20.

215. Ray A, Banerjee BD, Sen P. Modulation of humoral and cell-mediated immune responses by *Azadirachta indica* (Neem) in mice. *Indian J Exp Biol* 1996;**34**:698-701.

216. Ansari KU, Kasturi N, Tewarson SL, Singh S, Pandey RC. An experimental and clinical evaluation of immuno-modulating potential of *Azadirachta indica* (Neem) extract. *Indian J Clin Pharmacol Ther* 1997;**18**:82-4.

217. Ray A, Banerji BD, Koner BC, Sen P. Influence of *Azadirachta indica* (Neem) leaf extract on the immunotoxicity of stress and xenobiotics in experimental animals. *Indian J Pharmacol* 1997;**29**:38.

218. Mungantiwar AA, Nair AM, Shinde UA, *et al.* Effect of stress on plasma and adrenal cortisol levels and immune responsiveness in rats: modulation by alkaloidal fraction of *Boerhaavia diffusa*. *Fitoterapia* 1997;**68**:498-500.

219. De P, Dasgupta SC, Gomes A. Immunopotentiating and immunoprophylactic activities of Immune 21, a polyherbal product. *Indian J Pharmacol* 1998;**30**;163-8.

220. Sembulingam K, Sembulingam P, Namasivayam A. Effect of *Ocimum sanctum* Linn on noise induced changes in plasma corticosterone level. *Indian J Physiol Pharmacol* 1997;**41**:139-43.

221. Sur TK, Bhattacharya D. The effect of *Panax ginseng* and diazepam on brain and hypothalamic 5-hydroxytryptamine during stress. *Indian J Pharmacol* 1997;**29**:318-21.

222. Sarkar D, Sharma A, Talukder G. Clastogenic activity of pure chlorophyll and anticlastogenic effects of equivalent amounts of crude extract of Indian spinach leaf and chlorophyllin following dietary supplementation to mice. *Environ Mol Mutagen* 1996;**28**:121-6.

223. Sarkar S, Pranava M, Marita R. Demonstration of the hypoglycemic action of *Momordica charantia* in a validated animal model of diabetes. *Pharmacol Res* 1996;**33**:1-4.

224. Ghafoorunissa, Reddy V, Sesikaran B. Palmolein and groundnut oil have comparable effects on blood lipids and platelet aggregation in healthy Indian subjects. *Lipids* 1995;**30**:1163-9.

225. Ghafoorunissa. Nutritional and health implications of palm oil in Indian diets. *Indian J Med Res* 1995;**102**:233-40.

226. Kumar PD. The role of coconut and coconut oil in coronary heart disease in Kerala, South India. *Trop Doct* 1997;**27**: 215-7.

227. Dikshit M, Rastogi L, Shukla R, Srimal RC. Prevention of ischaemia-induced biochemical changes by curcumin and quinidine in cat heart. *Indian J Med Res* 1995;**101**:31-5.

228. Deshpande UR, Gadre SG, Raste AS, *et al.* Protective effect of turmeric (*Curcuma longa* L.) extract on carbon

tetrachloride-induced liver damage in rats. *Indian J Exp Biol* 1998;**36**:573-7.

229. Khosla P, Gupta DD, Nagpal RK. Effect of *Trigonella foenum* graecum (Fenugreek) on serum lipids in normal and diabetic rats. *Indian J Pharmacol* 1995;**27**:89-93.

230. Khan BA, Abraham A, Leelama S. Haematological and histological studies after curry leaf (*Murraya koenijori*) and mustard (*Brassica juncea*) feeding in rats. *Indian J Med Res* 1995;**102**:184-?.

231. Khan BA, Abraham A, Leelamma S. Hypoglycemic action of *Murraya koenigii* (curry leaf) and *Brassica juncea* (mustard): mechanism of action. *Indian J Biochem Biophys* 1995;**32**:106-8.

232. Khan BA, Abraham A, Leelamma S. Role of *Murraya koenigii* (curry leaf) and *Brassica juncea* (Mustard) in lipid peroxidation. *Indian J Physiol Pharmacol* 1996;**40**:155-8.

233. Sharathchandra JNN, Patel K, Srinivasan K. Digestive enzymes of rat pancreas and small intestine in response to orally administered mint (*Mentha spicata*) leaf and garlic (*Allium sativum*) oil. *Indian J Pharmacol* 1995;**27**:156-60.

234. Augusti KT. Therapeutic values of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.). *Indian J Exp Biol* 1996; **34**:634-40.

235. Sheela CG, Augusti KT. Effects of S-allyl cysteine sulfoxide isolated from *Allium sativum* Linn and gugulipid on some enzymes and fecal excretions of bile acids and sterols in cholesterol fed rats. *Indian J Exp Biol* 1995;**33**:749-51.

236. Sheela CG, Augusti KT. Antiperoxide effects of S-allyl cysteine sulphoxide isolated from *Allium sativum* Linn and gugulipid in cholesterol diet fed rats. *Indian J Exp Biol* 1995;**33**:337-41.

237. Mathew BC, Augusti KT. Biochemical effects of garlic protein diet and garlic oil on glycosaminoglycan metabolism in cholesterol fed rats. *Indian J Exp Biol* 1996;**34**:346-50.

238. Mathew BC, Daniel RS, Augusti KT. Hypolipidemic effect of garlic protein substituted for casein in diet of rats compared to those of garlic oil. *Indian J Exp Biol* 1996;**34**:337-40.

239. Rajasree CR, Rajamohan T, Augusti KT. Antiperoxide effect of garlic protein in alcohol fed rats. *Indian J Exp Biol* 1998;**36**:60-4.

240. Sharma SS, Kochupillai V, Gupta SK, Seth SD, Gupta YK. Antiemetic efficacy of ginger (*Zingiber officinale*) against cisplatin-induced emesis in dogs. *J Ethnopharmacol* 1997;**57**:93-6.

241. Sharma A, Mathur R, Dixit VP. Prevention of hypercholesterolemia and atherosclerosis in rabbits after supplementation of *Myristica fragrans* seed extract. *Indian J Physiol Pharmacol* 1995;**39**:407-10.

242. Ram A, Lauria P, Gupta R, Sharma VN. Hypolipidaemic effect of *Myristica fragrans* fruit extract in rabbits. *J Ethnopharmacol* 1996;**55**:49-53.

243. Khajuria A, Zutshi U, Bedi KL. Permeability characteristics of piperine on oral absorption - an active alkaloid from peppers and a bioavailability enhancer. *Indian J Exp Biol* 1998;**36**:46-50.

244. Karan RS, Bhargava VK, Garg SK. Effect of Trikatu (Piperine) of the pharmacokinetic profile of isoniazid in rabbits. *Indian J Pharmacol* 1998;**30**:254-6.

245. Prabhu MS, Platel K, Saraswathi G, Srinivasan K. Effect of orally administered betel leaf (*Piper betle* Linn.) on digestive enzymes of pancreas and intestinal mucosa and on bile production in rats. *Indian J Exp Biol* 1995;**33**:752-6.

246. Cherian KM, Gandhi VM, Mulky MJ. Toxicological evaluation of mowrah (*Madhuca latifolia Macbride*) seed meal. *Indian J Exp Biol* 1996;**34**:61-5.

247. Pal BC, Achari B, Yoshikawa K, Arihara S. Saponins from *Albizia lebbeck. Phytochem* 1995;**38**:1287-91.

248. Garai S, Mahato SB, Ohtani K, Yamasaki K. Dammarane-type triterpenoid saponins from *Bacopa monniera. Phytochem* 1996;**42**:815-20.

249. Garai S, Mahato SB, Ohtani K, Yamasaki K. Bacopasaponin D - a pseudojujubogenin glycoside from *Bacopa monniera. Phytochemistry* 1996;**43**:447-9.

250. Sivaprakasam P, Viswanathan S, Thirugnanasambantham P, *et al.* Przewalskinone-B from the stem bark of *Ochna obtusata. J Indian Chem Soc* 1997;**74**:165-?

251. Chaudhuri T, Das SK, Vedasiromoni JR, *et al.* Phytochemical investigation of the roots of *Camellia sinensis* L. (O. Kuntze). *J Indian Chem Soc* 1997;**74**;p166.

252. Sengupta S, Singh S, Sengupta LK, Bisen PS. Phytolectins: natural molecules with immense biotechnological potential. *Indian J Exp Biol* 1997;**35**:103-10.

253. Singh R, Gautam H, Jayaraman V, *et al.* Vegetative tissue lectins of peanut (*A. hypogaea*). *Indian J Biochem Biophys,* 1997;**34**:72-5.

254. Suresh B, Dhanasekaran S, Kumar RV, Balasubramanian S. Ethnopharmacological studies on the medicinal plants of Nilgiris. *Indian Drugs* 1994;**32**:340-52.

255. Bhandary MJ, Chandrashekar KR, Kaveriappa KM. Medical ethnobotany of the Siddis of Uttara Kannada district, Karnataka, *India J Ethnopharmacol* 1995;**47**:149-58.

256. Aswal BS, Goel AK, Kulshrestha DK, Mehrotra BN, Patnaik GK. Screening of Indian plants for biological activity: Part XV. *Indian J Exp Biol* 1996;**34**:444-67.

257. Johri RK, Singh C. Medicinal uses of Vernonia species. *J Med Aromatic Plant Sci* 1997;**19**:744-52.

258. Nazarine F, Anita F, Rataboli PV, *et al.* Pharmacological activities of extracts of some marine animals and plants on isolated tissues of the guinea pig. *Indian J Marine Sci* 1998;**27**:499-501.

259. Mazumdar UK, Gupta M, Maiti S. Effect of petroleum ether extract from *Hygrophila spinosa* on hematological parameters and hepatorenal functions in mice. *Indian J Exp Biol* 1996;**34**:1201-3.

260. Sarathchandra G, Balakrishnamurthy P. Pertubations in glutathione and adenosine triphosphatase in acute oral toxicosis of *Cleistanthus collinus* : An indigenous toxic plant. *Indian J Pharmacol* 1997;**29**:82-5.

261. Biswas NR, Sen S, Singh S, *et al.* Sub-acute toxicity study of a polyherbal drug (Prostina(R)) in rats. *Indian J Pharmacol* 1998;**30**:239-44.

262. Samudralwar DL, Garg AN. Minor and trace elemental determination in the Indian herbal and other medicinal preparations. *Biol Trace Elem Res* 1996;**54**:113-21.

263. Singh V, Garg AN. Availability of essential trace elements in Ayurvedic Indian medicinal herbs using instrumental neutron activation analysis. *Appl Radiat Isot* 1997;**48**:97-101.

264. Rajurkar NS, Pardeshi BM. Analysis of some herbal plants from India used in the control of diabetes mellitus by NAA and AAS techniques. *Appl Radiat Isot* 1997;**48**:1059-62.

265. Uchil D, Rege NN, Dahanukar SA. Modern correlates for Ayurvedic quality control tests for Tamrabhasma. *Indian Drugs* 1996;**33**:384-9.

266. Gupta SS. Prospects and perspectives of natural plant products in medicine. *Indian J Pharmacol* 1994;**26**:1-12.

267. Vaidya AB, Antarkar VDS. New drugs from medicinal plants: opportunities and approaches. *J Assoc Phys India* 1994; **42**:221-8.

268. Singh A, Handa SS. Hepatoprotective activity of *Apium graveolens* and *Hygrophila auriculata* against paracetamol and thioacetamide intoxication in rats. *J Ethnopharmacol* 1995;**49**:119-26.

269. Gulati RK, Agarwal S, Agrawal SS. Hepatoprotective studies on *Phyllanthus emblica* Linn. and quercetin. *Indian J Exp Biol* 1995;**33**:261-8.

270. Gupta NK. Modification of radiation induced changes in murine hepatic lipid profiles by garlic (*Allium sativum* Linn.) unsaturated oils. *Indian J Exp Biol* 1996;**34**:851-3.

271. Mukherjee S, Sur A, Maiti BR. Hepatoprotective effect of *Swertia chirata* on rat. *Indian J Exp Biol* 1997;**35**:384-8.

272. Jayasekhar P, Mohanan PV, Rathinam K. Hepatoprotective activity of ethyl acetate extract of *Acacia catechu. Indian J Pharmacol* 1997;**29**:426-8.

273. Mitra S, Sur RK. Hepatoprotection with *Glycosmis pentaphylla* (Retz). *Indian J Exp Biol* 1997;**35**:1306-9.

274. Sharma M, Pillai KK, Husain SZ, Giri DK. Protective role of propolis against alcohol - carbon tetrachloride-induced hepatotoxicity in rats. *Indian J Pharmacol* 1997;**29**:76-81.

275. Subramoniam A, Evans DA, Rajasekharan S, Pushpangadan P. Hepatoprotective activity of *Trichopus zeylanicus* extract against paracetamol-induced hepatic damage in rats. *Indian J Expt Biol* 1998;**36**:385-9.

STATUS REPORT ON NEUROPHARMACOLOGY

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In surveying the work done in the field of neuropsychopharmacology, this review has been divided into the following seven sections: 1) Neurotransmitters, neuromodulators, receptors and ion channels, 2) Behavioural research, 3) Neuropharmacological studies, 4) Central control of peripheral functions, 5) CNS active synthetic drugs, 6) CNS active plant derived drugs, 7) Miscellaneous research on CNS active drugs.

1. Neurotransmitters, Neuromodulators, Receptors and Ion Channels

1.1. Catecholamines

The role of dopaminergic mechanisms in the regulation of stress responses has been studied in experimental animals. The complex dopaminergic mechanisms involved in the regulation of visceral, endocrinological and immune responses during stress were discussed[1]. A differential dopamine receptor involvement during stress- induced ulcer formation in rats has been reported. The gastric cytoprotective role for dopamine (DA) and dopaminergic (DA_1-DA_2) receptor interactions occurring during dopaminergic regulation of gastric mucosal integrity during stress has been explored [2].

Studies have indicated the role of DA in drug induced stereotyped behaviour in rats. dl - Pentazocine produced stereotyped behaviour in rats by releasing DA from nigrostriatal and mesolimbic dopaminergic neurons with resultant activation of post synaptic striatal and mesolimbic D_2 DA receptors by the released DA[3]. Pentazocine and metoclopramide have been shown to induce catalepsy in mice. Pentazocine induced catalepsy was mediated through opiod mechanism rather than D_2 DA blockade[4] whereas, metoclopramide induced catalepsy was found to be mediated through selective blockade of D_2 DA receptor at the post synaptic level[5]. In a study, it was found that DA is metabolized preferentially, if not exclusively by MAO-A in the monkey brain[6].

1.2. Serotonin

Central serotonergic mechanisms involved in the baclofen-induced catatonia have been studied using serotonergic agents[7]. Central serotonergic system also been reported to modulate the pentazocine-induced catalepsy in mice[8]. In another study the differential effects of serotonergic agents on different components of amphetamine induced stereotypy have been reported[9].

5-HT antagonistics were found to inhibit the 5-HT-induced aggregation in isolated skin melanophores of wall lizard (*Hemidactylus flaviviridus*). Coexistence of more than one type of 5-HT receptors, which mediate the aggregatory responses of the lizard melanophores to 5-HT has been indicated[10].

In a study on 5-HT_3 receptors, ondansetron, a 5-HT_3 receptor antagonist showed anti-anxiety activity in a novel animal model of anxiety[11,12]. 5-HT receptor systems, particularly 5-HT_3 receptor, may play a modulating role in learning and memory phenomena in animal models[13]. 5-HT_3 receptors play an important role in morphine discontinuation phenomena[14]. Sumatriptan, a novel 5-HT_{1D} receptor agonist and anti-migraine drug was found to show antinociceptive effect in mice by possibly releasing acetyl choline[15].

The concentration of monoamines and their metabolites were investigated in hypothalamus, hippocampus, striatum, pons-medulla and in frontal cortex region of brains of 3 day diabetic and insulin treated male Wister rats. The results indicated that norepinephrine content increased in the hypotha-lamus, striatum and pons-medulla and the concentration of its metabolite, methylhydroxyphenylglycol was decreased in hypothalamus and pons-medulla in diabetic rats. The concentration of the serotonin was higher in all the brain regions except striatum. Levels of 5-HT metabolite, 5-hydroxyindole acetic acid, was also higher in all the five regions investigated in diabetic rats. Decreased dopamine and its metabolite, dihydroxyphenyl acetic acid, levels were observed in hypothalamus, striatum and cortex in diabetic conditions. Insulin treatment STZ diabetic rats reversed the brain monoamines and monoamine metabolites perturbations[16].

1.3. Cholinergic receptors

A simple analytical method has been devised for estimation of RBC and plasma cholinesterase using cysteine as substrate. The colour reaction was stable and reliable. The variation in species has also been determined[17]. This method has been successfully extended to organophosphorus poisoning in human studies[18,19].

1.4. GABA

In a study the various neuro-excitatory components of diazepam withdrawal reactions and correlation of them with the brain levels of GABA have been discussed[20].

International Union of Pharmacology (IUPHAR) through its nominated Sub-committee on various neurotranmitter receptors has classified various receptor types according to IUPHAR guidelines. The opioid receptors classification and nomenclature has been done by Drs Raghubir and Dhawan along with other Committee members. These are now designated as OP1 (δ), OP2 (κ) and OP3 (μ) opioid receptors[21]. The receptor subtypes, their agonists antagonists, radioligands their agonists, antagonists, radioligands, tissue distribution and functions have been recently demonstrated[22].

1.5. Tribulin and isatin

Tribulin has been proposed to be an endocoid marker of anxiety and stress. It has two major components, one being identified as isatin (2,3-dioxindole) having MAO-B inhibitory and benzodiazepine receptor inhibitory activity. The pharmacology of isatin and tribulin has been comprehensively reviewed[23]. The role of tribulin as an anxiogenic endocoid was demonstrated by the increase of rat brain tribulin activity in anxiogenic responses induced by several anxiogenic agents, including pentylenetetrazol, yohimbine[24], caffeine[25], scorpion venom[26], bradykinin[27], arginine-vasopressin[28] and methylenedioxymethamphetamine (Ecstasy)[29] and in anxiety following addictive drugs (morphine, lorazepam, alcohol, nicotine and cannabis) withdrawal[30], in this species. The anxiogenic response of isatin, reported earlier in rodents, was confirmed in Rhesus monkeys[31]. Isatin has recently been shown to inhibit the binding of atrial natriuretic peptide (ANP) to its specific receptors in several mammalian brain areas. This was confirmed when isatin was found to inhibit the anxiolytic[32], anticonvulsant[33] and memory facilitating effects[34] of centrally (*i.c.v.*) administered ANP. Isatin has been shown to disrupt the memory engram in rats[35]. The likely biosynthetic pathway for the synthesis of endogenous isatin was proposed following elaborate chemical reactions *in vitro* and *in vivo* [36]. Very early studies, conducted more than four decades ago, indicated that isatin has significant anticonvulsant activity. Behavioural studies[25] have shown that the anxiogenic action of isatin is lost and replaced by central sedation on dose increment, possibly due to accumulation of some unknown metabolite. In keeping with this theory, isatin was found to have a dose-related convulsant and anticonvulsant activity. Convulsions were induced in rodents by anxiogenic doses of isatin, whereas, an anticonvulsant effect was seen in higher doses[37]. Apart from its effect on ANP receptors, isatin appears to also stimulate 5-HT$_3$ receptors, which being heteroreceptors appear to activate the mesolimbic dopaminergic system. This 5-HT$_3$-dopamine nexus, in the action of isatin, was demonstrated by elaborate pharmacological and neurochemical studies[38].

1.6. Atrial natriuretic peptide (ANP)

ANP, administered *i.c.v.*, induced significant anxiolytic activity in rodents[32] which was not mediated through benzodiazepine receptors. In anxiolytic doses, ANP had a memory facilitating[34] and anticonvulsant activity[33].

1.7. Neurosteroids

The role of neurosteroids in the brain function are on the ascend and are proposed to play an important role in homeostatic mechanisms of the body through neuroendocrine interactions in a variety of conditions such as stress, anxiety, epilepsy, aggression, learning and memory, sexual and sleep related disorders[39]. The studies on immobilization and hypoxic stress suggested the pivotal anti-stress role of neurosteroids[40,41]. Studies on dependence and tolerance by morphine and benzodiazepines suggested the role of dihydropyridine-sensitive Ca^{2+} channels and mitochondrial DBI receptors in the anti-addictive action of neurosteroids and further potential utility of specific neurosteroids in its treatment[42-44]. Detailed investigations on neurosteroids on new animal models of anxiety demonstrated the differential effect of neurosteroids in anxiety[45,46]. Many of the neurosteroids induce hyperphagic effect by acting on $GABA_A$ and mitochondrial DBI receptors[47]. Chronic studies have shown that oestrous cycle and sex modified the effect of both neurosteroids and benzodiazepines[48]. Neurosteroids modulate learning and memory by acting at sigma receptors and neuronal nitric oxide pathway[49,50]. Neuronal nitric oxide pathway plays an important role in the behavioural effects of ACTH[51]. Neurosteroids were shown to have antidepressant and proconvulsant effects[52,53].

1.8. Melatonin

In a study on CNS action of melatonin, it was speculated that some of the actions of melatonin involve $GABA_A$ receptors distinct from the benzodiazepine site in the CNS[54,55]. In addition, mysterious decrease of melatonin in depression and its role in affective disorders was reported[56]. In an immunological study, the potential role of melatonin in regulating the activation of the antigen -specific T cell response was suggested[57]. Melatonin possibly acts by selectively activating a Th-2-like immune response.

1.9. Angiotensin receptors

Emerging evidence has suggested the existence of a separate local Renin Angiotensin System (RAS) in the brain. A recent study on losartan, an AT1 receptor antagonist, has demonstrated its nootropic activity and further suggested the role of cholinergic system in facilitating its cognitive enhancing effect[58]. Losartan was also found to modulate dopaminergic system in the brain[59].

2. Behavioural Research

2.1. Anxiety

Scorpion envenomation is associated with severe anxiety and panic reactions. Central (*i.c.v.*) administration of venom of *Mesobuthus tamulus* induced marked anxiety in rats[26]. It was postulated that the anxiogenic response may be due to the release of bradykinin. This was confirmed when *i.c.v.* administered bradykinin was found to have a qualitatively similar anxiogenic activity in this species[27]. Likewise, the designer drug of abuse, methylenedioxyamphetamine, widely used as recreational agent and popularly known as Ecstasy, was found to induce a serotonin-mediated anxiogenic response[29], confirming the clinical symptoms of the drug which include severe anxiety, agitation and panic reaction. Caffeine was also shown to induce an anxiogenic effect in rats which diminished on subchronic treatment over 2-3 weeks. Cessation of treatment was associated with withdrawal anxiety[25]. The study confirmed the clinical evidence of caffeine-induced anxiety, tolerance to anxiety on continued use and withdrawal symptoms in chronic caffeine-containing beverage users. A number of hallucinogens, which are methylated tryptamine derivatives, exhibited a serotonin-mediated anxiogenic effect in sub-hallucinogenic doses, confirming clinical symptoms of severe agitation and anxiety prior to the hallucinogenic state[38].

In a study conducted in streptozotocin (STZ)-induced diabetic rats, it was shown that diabetic rats exhibited significantly more anxiety than their euglycaemic counterparts. The anti-anxiety effect of diazepam was less marked in STZ-diabetic rats[60]. Prenatal administration of diazepam was found to be associated with augmented anxiety in the offsprings[61]. Following the observations that ANP, which induces diuresis, exhibits anxiolytic activity, and arginine-vasopressin (AVP), an antidiuretic with anxiogenic activity, it was suggested that these two peptides may be involved in acute water intoxication in some psychiatric patients. It was further noted that isatin, a putative endogenous marker of anxiety, which had an antidiuretic effect, could antagonize the anxiolytic effect of ANP and potentiate that of AVP. These findings, in concert with earlier data, have led to the postulate that ANP, AVP and isatin may be involved in water balance and imbalance in psychiatric states[62,63].

A primate model of anxiety was developed[64,65]. Fear potentiated poststartle activity in neonatal clomipramine treated rats was investigated[66].

2.2. Learning and memory

The effect of DSP-4 -induced depletion of central norepinephrine (NE) on retrieval of aversive memories in rats was studied. The results indicated that decreased NE in striatum coincided with the memory changes suggesting that NE innervation to striatum may participate in the retrieval process[67]. The role of muscarnic receptor in the retrieval of shock motivated brightness discrimination responses was described[68]. Rate of administration of ECS[69,70], calcium channel blockers, antihypertensive drugs[71] and BR-16A[72,73] have been tested in ECS induced antrograde and retrograde amnesia.

In a study, changes in learning and memory, acetyl cholinesterase activity and monoamines in the brain after administration of carbamazepine in rats were discussed[74]. Studies were conducted to evaluate the effects of corticotrophin releasing factor (CRF), α-helical CRF, ACTH, ACTH 4-10 and arginine-vasopressin on aversive memory retrieval in rats using T-maze. The results suggested the possible involvement of central CRF, ACTH and vaso-pressinergic mechanisms in the differential enhancement of memory of helpless conditions[75-77].

A study reported that centrally administered oxytocin failed to alter the appetitive and aversive memory retrieval process[78]. Effect of p-chlorophenyl alanine-induced depletion of brain serotonin on retrieval of appetitive and aversive memories was studied. It was found that depletion of 5-HT failed to produce a differential improvement of aversive memory retrieval. However, it produced anxiolytic effect as evidenced from the enhanced traversing behaviour in T-maze and increased central activity in open field[79].

Effects of some psychotropic agents (chlorpromazine and haloperidol) on cognitive functions in rats were studied. It was observed that all these psychotropic agents inhibited the active avoidance response and differentially affected the maze task performance[80]. A relationship among the swim stress, cholinergic activity and avoidance memory process has been established for the first time in rats[81]. Chronic phenytoin treatment produced impairment of learning and memory with associated changes in brain acetylcholine esterase (AChE) and monoamine levels[82].

3. Neuropharmacological Studies

3.1. Convulsions

A comparison of state dependent effect of phenytoin and phenobarbital at different doses was studied in rats, using inhibitory avoidance and food motivated T-maze task[83]. The effect of buspirone on chemically-induced seizures in mice was studied. Buspirone exhibited pro-convulsive action on PTZ seizures possibly mediated through α_2-adrenorece-ptors[84]. In a study, the anticonvulsant effects on seizure threshold have been determined using ramp generated cortical stimulation in rats[85]. The influence of adenosine, dipyramide, adenosine antagonists and antiepileptic drugs on EEG after discharge following cortical stimulation has been discussed[86].

The search for therapeutic approaches to epilepsy has been based on animal seizure models[87,88]. In a study on lithium-pilocarpine-induced status epilepticus, involvement of different neurotransmitter systems such as GABA, excitatory amino acids, nitric oxide, dopamine and adenosine were suggested[89,90]. It was experimentally proved that calcium channels play an important role in the neuronal mechanism of status epilepticus. Clozapine, the prototype of atypical antipsychotic was found to protect animals from pentylenetetrazol- and pilocarpine-induced convulsions[91]. A new animal model for status epilepticus, in which development of self-sustaining limbic status epilepticus by continuous ventral hippocampal stimulation followed by low dose pilocarpine in rats was established[92].

3.2. Antipsychotics

The pharmacological basis of atypical antipsychotic drugs has been the target of intensive study since the significance of clozapine was first appreciated. Various hypothesis have been promulgated on this subject[93]. Recently it was suggested that partial agonistic action of clozapine and olanzapine at dopamine D_2 receptors could contribute to its atypical clinical profile[94,95]. Another factor is the anatomical specificity within the brain that may account for the difference in acute extrapyramidal side effects liability between atypical and typical antipsychotic drugs[96]. It was recently suggested that 5-HT_{2A} receptors contribute to the action of dizocilpine, an excitatory amino acid antagonist[97]. The role of dopamine receptors in the central actions of dizocilpine was recently supported by studies on feeding behaviour[98]. It was established that the antimuscarnic actions of clozapine could contribute to its cognitive impairment effects in animal models of learning and memory[99,100].

3.3 Sleep disorders

The changes in sleep architecture, heart rate and respiratory rate to hyoscine butyl bromide (HBB), a peripherally acting anticholinergic drug atropine were studied[101]. Another study discussed the effect of hyoscine butyl bromide and atropine on heart rate during nocturnal sleep[102]. Effect of karnatic music raga *"Neelambari"* on sleep architecture was evaluated[103]. Guidelines to alert the researchers and clinicians, interested in setting up sleep labs in India and other developing countries were reported[104]. A short note on sleep medicine as an emerging medical specialty has been published[105].

3.4. Analgesic and antipyretic studies

Antinociceptive activity of a noval meta enkephalin analogue which is a potent systemic μ opioid receptor agonist was reported[106]. Enkephalin antisense peptide approach may be useful in SAR studies of biologically active endogenous enkephalins[107]. Efforts were also made to synthesize tetra-peptide and hepta-peptides analogous to dermorphin to obtain μ opioid receptor selective analgesics. A dermorphin tetra-peptide was about 3 fold[108] and a heptapeptide was twice active analgesic as compared to dermorphin[109]. Further, anti-inflammatory and analgesic activity of various thiozolines was also observed[110].

A mild electrical stimulation of tiny sites in pretectal nucleus of rat evokes analgesia of long duration. Central cholinergic, adrenergic and opioidergic mechanisms appear to be involved in the pretectal stimulation produced analgesia (SPA)[111]. Further microinjection of 5-HT and opioid into pretectal nucleus produced marked analgesic response[112]. There is strong evidence to suggest that nitric oxide (NO) is involved in pretectal SPA[113]. Various neurotransmitters like 5-HT, NE and endogenous enkephalins seem to be modulate analgesic effects of opioid drugs[114].

4. Central Control of Peripheral Functions

4.1. Nitric oxide, vascular permeability and central CV regulation

Studies have been conducted to evaluate the role of l-arginine EDRF/ NO pathway in normal and chloralose anaesthetized cats[115]. The role of nitric oxide in modulation of blood brain barrier permeability in normal and infected rats has been reported[116-119]. A study explained the interrelationship between blood pressure level and nitric oxide synthase activity in rats[120].

4.2. Modulation of body temperature

Modulation of the body temperature by endogenous peptide, cyclo (His-Pro), at hot ambient temperature, and in cold induced hypothermia was studied in dessert rats (*Mastomys natalansis*)[121,122]. The peptide also attenuated the alcohol induced hypothermia[123].

5. CNS Active Synthetic Drugs

Synthetic compounds, 2-N(substituted) aminopyrido [1,2,9] pyrimidin-4-oxo-1-H-3-carbonitriles, 2-(N-substituted aminoethyl)-1,3-diphenyl-3-(4-methoxy phenyl)-propane-1-3-diones and N-(4-benzylidine-2-methyl phenyl-oxo-imidazoline-1-yl) acetamide and their (4-substituted phenyl) -azo analogs were reported to have analgesic activity[124-126].

6. CNS Active Plant Derived Drugs

Several Indian medicinal plants were investigated for their CNS effects during the period of survey. The results of these investigations have been categorized according to the major pharmacological property reported.

6.1. Antidepressant activity

Hypericum perforatum (St. John's Wort) has been in use in Europe as a herbal remedy for melancholia and related clinical disorders. An extensive intensive investigation showed that the plant extracts had significant antidepressant activity against several animal models of clinical depression[127]. It was shown that the activity was due to hyperforin, rather than the earlier claims made for another chemical moiety, hypericin[128].

6.2. Anxiolytic activity

A standardized extract of *Bacopa monniera* (Brahmi), based on the projected active principles Bacoside A and B, was found to have significant anxiolytic activity in animal models of clinical anxiety, in doses earlier reported to have a memory-facilitating effect[129]. The applied importance of the work lay in the fact that all anxiolytics in clinical use are known to have adverse effect on memory. *Azadirachta indica* (Neem) leaf extracts were reported to induce anxiolysis in rodents[130]. Following extensive chemical and pharmacological investigations on the *Indian Ginkgo biloba*[131], which represented a concerted effort to dispell the belief that *G.biloba* was not indigenous to India, the ginkgolic acid conjugates were found to have anxiolytic activity[132].

6.3. Learning and memory

Withania somnifera (Aswagandha), an Ayurvedic *medhya rasayana* (memory-facilitating drug), was shown to attenuate amnesic effects in animal models of Alzheimer's disease by reversal of cholinergic dysfunction induced by the amnesic agents[133]. Ayurvedic formulations, based on *W. somnifera*, induced a similar amnesia-reversal effect in rats[134,135].

The oil of *Celustrus paniculatus* was reported to stimulate the intellect and memory. The acetone soluble fraction of petroleum ether extract of *Lawsonia* inermis exhibited prominent nootropic activity mediated by 5-HT and NA[136].

6.4. Antioxidant activity

Glycowithanolides from *W. somnifera*[137], standardised extracts of *Ocimum sanctum* (Tulsi)[138], and Shilajit[139,140], were shown to exert significant oxidative free radical scavenging activity in different rat brain areas like frontal cortex, striatum and hippocampus.

6.5. Antistress activity

A compound herbal formulation, incorporating *W. somnifera*, Shilajit and *Emblica officinalis*, was shown to attenuate the adverse effects of acute stress[141]. A gem containing Unani formulation, Jawahir Mohra, has been found to have antistress activity[142].

6.6. Neuropharmacological studies

Panex ginseng, a widely used antistress adaptogenic agent, was investigated for its general neuropharmacological profile of activity[143].

Methanolic extract of *Cassia fistula* significantly potentiated the sedative actions of sodium pentobarbitone, diazepam, meprobamate and chlorpromazine, and analgesic responses of morphine and pethidine. A general depressant activity was reported from behavioural studies on mice[144]. The petroleum ether extract of *Vitex negundo*, is reported for analgesic and anticonvulsant activity at higher doses in experimental animal models[145].

The alcoholic extract of *Nardostachys jatamansi* was reported to posses anti-convulsant effects in MES induced seizures and the effect of the extract on certain neurotransmitter levels was also studied[146].

Calcined gold preparations, Ayurvedic swarna bhasma and Unani Kushta Tilaka were reported to posses analgesic activity[147]. These preparations also showed nootropic, glycogen sparing, anticateleptic and adaptogenic activities[148]. Bacosine, a new diterpine isolated from the ariel parts of *Bacopa monnieri*, has been found to have analgesic activity. However, it did not affect pentobarbitone induced sleeping time, conditioned avoidance response, spontaneous motor activity and haloperidol induced catalepsy[149].

Analgesic activity of *Azadirachita indica* (Neem) was reported in different animal models[150]. Two calcined Unani preparations Kushta Marjan (from coral) and Kushta Khar (from courie) were reported to have non-narcotic analgesic effects[151].

Methanolic extract of *Moringa oleifera* root showed 70 % protection against strychnine induced convulsions, whereas, petroleum ether extract of *Mucuna pruriens* and *Vitex negundo* showed 50 % protection[152].

Extensive studies on convulsions showed that *Withania somnifera* root extract is effective against PTZ - induced kindiling epilepsy in mice and amygdaloid kindiling in rats[153,154] and lithium-pilocarpine-induced seizures[155]. Protective effect of *W.somnifera* root extract on electrographic activity in a lithium-pilocarpine model of status epilepticus was demonstrated recently[156]. Two herbal formulations such as BR-16A and Siotone® granules were found to be anticonvulsant in animal models[157,158]. BR-16A reverses benzodiazepine induced withdrawal in mice[159,160]. *W. somnifera* was found to inhibit morphine tolerance and dependence in mice[161]. An extensive study on Siotone® granules demonstrated its CNS depressant effects as well as its beneficial effects in anxiety and cognition in animals[162]. Two herbal formulations BR-16A and Siotone® granules, *W. somnifera* and evening primrose oil were found to reverse forced swimming -induced chronic fatigue syndrome in mice[163]. Another herbal formulation UL-409 was found to protect gastric ulcer in rats[164]. Dopamine mediated hyperlocomotor activity by spirulina was suggested recently[165].

6.7. Effect on central neurotransmitters

In a first report of its kind, the effects of standardized extracts of Shilajit and *W. somnifera* were subjected to an autoradiographic study in different rat brain regions[166]. Treatment with Shilajit or a defined extract from *W.somnifera* affected neither $GABA_A$ and benzodiazipine receptor binding nor NMDA and AMPA glutamate receptor subtypes in any of the cortical or sub cortical regions but increased M_2 receptor binding. The drug-induced increase in cortical muscarnic acetylcholine receptor capacity might partly explain the cognition-enhancing and memory-improving effects of extracts from *W. somnifera* observed in animals and humans.

Ginkgolic acid conjugates from the Indian variety of G.biloba were shown to have significant effects on rat brain monoamines and their major metabolites, which could help explain the observed neuropharmacological effects of these active principles[167]. The acute administration of *Wrightia tinctoria* (*Wt*) (leaves) methanolic extractives differentially modulated neurotransmitters and their metabolites which can explain the behavioural effects of *Wt*, namely anxiolytic and antidepressant effects[168].

7. Miscellaneous research on CNS active drugs

7.1. Neurotoxicity

F.moniliformae mycotoxins (mainly zearalenone, diacetoxyscirpenol and T-2 toxins) exhibited a neuropharmacological profile qualitatively similar to that induced by the non-selective monoamine oxidase inhibitor, nialamide. A wide range of *in vivo* techniques were utilized to compare nialamide with the toxin[169]. On the contrary, mycotoxins from *F. oxysporum* (mainly fusaric acid, T-2 toxin and diacetoxyscirpenol) exhibited a profile of activity qualitatively similar to central dopamine receptor agonists. The effects were postulated to be due to fusaric acid which inhibits dopamine-β-hydroxylase[170]. In a late study[171], neurochemical investigations were conducted in different regions of rat brain and the findings were consonant and supportive with the pharmacological studies.

Transplantation of cerebellar cells reversed the neurochemical and behavioural deficits induced by acrylamide[172]. It was observed that the protein deficiency makes young animals vulnerable to the neurochemical toxicity of styrine[173].

Unilateral injection of 6-hydroxy dopamine (6-OHDA) in to the caudate nucleus of rat caused degeneration of dopaminergic neurons. Transplantation of embryonic neuronal tissue had ameliorated the neurochemical, behavioural and electrophysiological alterations produced by 6-OHDA[174].

Effects of lead exposure and iron deficiency on polyamine levels in neuronal and glial cells of cerebellum and hippocampus have been investigated in weaned rats. Results from these study indicated a depressed polyamine pathway leading to a impaired neuronal development in the neonatal rats[175].

In a study, cadmium induced alterations in blood brain barrier permeability and its possible correlation with decreased microvessel antioxidant potential in rat has been demonstrated[176]. A correlation between the severity of symptoms in organophosphprus poisoning and cholinesterase activity in human RBCs and plasma was observed[19].

Assessment of comparative restoration of behavioural and neurochemical deficits in trimethyltin chloride treated rats following single and multiple site fetal hippocampal transplantation was carried out. The results suggested that multiple site transplantation provides better restoration on long term basis[177]. The effect of fetal neural transplantation on trimethyltin induced cholinergic and serotonergic functional damage was studied. The study concluded co-grafting technique could provide a better restoration of functional deficits when more than one type of neuronal population is damaged[178]. A deltamethrin formulation (Decis) produced a significant neurochemical and neuromorphological changes which may culminate in perturbed synaptic function[179]. Use of toxic metals in Indian systems of medicine; Ayurveda, Siddha and Unani-Tibb has been reviewed[180].

There is strong evidence to suggest that microinjection of foetal neural tissue could significantly improve neurochemical electrophysiological and behavioural deficit caused by lesioning of caudate nucleus[181]. Topical application of NO donor, SNP to exposed ventral surface of medulla in cat produced hypotension and bradycardia which were reversed by pretreatment with methylene blue[182].

7.2. Drug Interaction

Interaction of ciprofloxacin with diclofenac and paracetamol in relation to its epileptogenic effect was studied. Epileptogenic effect produced by ciprofloxacin was potentiated by diclofenac but not by paracetamol[183]. A study reported a synergistic effect of flunarizine and sodium valproate on seizure threshold elicited by cortical stimulation in conscious rats[184]. Additive anti-convulsant effect of flunarizine and sodium valproate on electroshock and chemoshock induced seizures has been reported[185].

7.3. Behavioural teratology

A welcome aspect was the initiation of investigations on behavioural teratology for the first time in this country. A single prenatal exposure of rat dams to haloperidol during the critical period of gestation when neural cell proliferation occurs, appeared to leave a lasting imprint on the offspring in terms of abnormal emotional states, including anxiety and depression[186,187]. Prenatal administration of phenobarbitone induced cognitive deficits in the rat offspring's, and potentiated cognitive deficits induced by undernutrition in this species[188].

7.4. Methodology

The methods used for the experimental evaluation of anxiolytics[189] and antidepressants[190], in rodents, have been extensively discussed in terms of their predictive, construct and face validity.

8. Reviews

Several excellent reviews were published. They include discussions on novel targets for the development of newer analgesics based on advances in the knowledge of pain mechanisms and subtypes of the cyclo-oxygenase enzyme[191,192]. The rational management of postoperative pain in children[193,194], advances in the management of senile dementia[195], the use of selective serotonin reuptake inhibitors in psychiatry[196] and sumatriptan, a new drug for migrain[197] were the subjects of other reviews. Some recent developments in the blood brain barrier has been reviewed[198].

REFERENCES

1. Puri S, Ray A, Chakravarti AK, Sen P. Role of dopaminergic mechanisms in the regulation of stress responses in experimental animals. *Pharmacol Biochem Behav* 1994; **48**:53-6.
2. Puri S, Ray A, Chakravarti AK, Sen P. A differential dopamine receptor involvement during stress ulcer formation in rats. *Pharmacol Biochem Behav* 1994;**47**:749-52.
3. Bhosale BB, Balsara JJ, Jadhav JH. Involvement of dopaminergic mechanisms in racemate pentazocine induced stereotyped behaviour in rats. *Indian J Physiol Pharmacol* 1996;**40**:271-73.
4. Bhosale BB, Balsara JJ, Jadhav JH. Effects of pentazocine on dopamine dependent behaviours in mice. *Indian J Exp Biol* 1997;**35**:448-56.
5. Bhosale BB, Balsara JJ, Gaonkar RK, Bhosale BB, Gupta SK. Dose -dependent response of central dopaminergic systems to metoclopramide in mice. *Indian J Exp Biol* 1997; **35**:618-22.
6. Lakshmana MK, Rao BSS, Dhingra NK, Ravikumar R, Sudha S *et al.* Role of monoamine oxidase type A and B on the dopamine metabolism in discrete regions of the primate brain. *Neurochemical Res* 1998;**2B**:1033-9.
7. Kasture SB, Mandhane SN, Chopde CT. Baclofen -induced catatonia:modification by serotonergic agents. *Neuropharmacol* 1996;**35**:595-8.
8. Umrani DN, Kasture SB, Kasture US. Effects of drugs influencing central serotonergic mechanisms on pentazocine-induced catalepsy in mice. *Indian J Exp Biol* 1998; **30**:34-8.
9. Kandi CS, Metkar BR, Kasture US, Kasture SB. Effect of serotonergic agents on amphetamine induced stereotypy. *Indian J Pharmacol* 1998;**30**:334-38.
10. Ovais M, Parveen S, Gaur A. Inhibition of 5-HT induced aggregation in melanophores of the wall lizard (*Hemidactylus flaviviridis*) by specific antagonist in *in vitro. Indian J Exp Biol* 1994;**32**:513-4.
11. Kulkarni SK, Roychoudhury M. 5-HT_3 receptors. A review of their pharmacologic and therapeutic aspects. *Drugs of Today* 1996;**32**:515-28.
12. Roychoudhury M, Kulkarni SK. Antianxiety profile of ondansetron, a selective 5-HT_3 antagonist, in a novel animal model. *Meth Find Exp Clin Pharmacol* 1996;**19**:107-11.

13. Roychoudhury M, Kulkarni SK. Effect of ondansetron on short term memory retrieval in mice. *Meth Find Exp Clin Pharmacol* 1996;**19**:43-6.

14. Roychoudhury M, Kulkarni SK. Prevention of morphine discontinuation phenomenon in mice by ondansetron, a selective 5-HT_3 antagonist. *Meth Find Exp Clin Pharmacol* 1996;**18**:577-83.

15. Jain NK, Kulkarni SK. Antinociceptive effect of sumitriptan in mice. *Indian J Exp Biol* 1998;**36**:973-9.

16. Ramanathan M, Jaiswal AK, Bhattacharya SK. Brain monoamines and metabolites during early streptozotocin - induced diabetes in rats. *Biogenic Amines* 1997;**13**:55-63.

17. Venkataraman BV, Nagarani MA. Species variation in the specificity of cholinesterases in human and rat blood samples. *Indian J Physiol Pharmacol* 1994;**38**:17-9.

18. Venkataraman BV, Nagarani MA. Andrade C, Joseph T. Correlation of time course of blood cholinesterase activity and toxic manifestations of acute methyl parathion in antidote treated rats. *Indian J Physiol Pharmacol* 1994;**38**:20-2.

19. Bobba R, Venkataraman BV, Pais P, Joseph T. Correlation between the severity of symptoms in organophosphorus poisoning and cholinesterase activity (RBC and plasma) in human. *Indian J Physiol Pharmacol* 1996;**40**:249-52.

20. Singh H, Goyal BL, Singh P. Study of neuro-excitatory features during diazepam withdrawal reactions and their correlation with brain gamma amino butyric acid levels. *Indian J Pharmacol* 1998;**30**:267-70.

21. Dhawan BN, Casselin F, Raghubir R, Resine T, Bradley PB, Portoghese PS, Hamon M. International Union of Pharmacology. XII Classification of Opioid Receptors. *Pharmacol Rev* 1996;**48**:567-92.

22. Dhawan BN, Raghubir R, Hamon M. Opioid receptors, IUPHAR Compendium of receptor characterization and classification. IUPHAR Media, London, UK 1998; 218-24.

23. Glover V, Bhattacharya SK, Chakrabarti A, Sandler M. The psychopharmacology of isatin: a brief review. *Stress Med* 1998;**14**:225-9.

24. Bhattacharya SK, Chakrabarti A, Sandler M, Glover V. Effects of some anxiogenic agents on rat brain monoamine oxidase (MAO) A and B inhibitory (tribulin) activity. *Indian J Exp Biol* 1996;34:1190-3.

25. Bhattacharya SK, Satyan KS, Chakrabarti A. Anxiogenic action of caffeine: an experimental study in rats. *J Psychopharmacol* 1997;**11**:219-24.

26. Bhattacharya SK. Anxiogenic action of centrally administered scorpion (*Mesobuthus tamulus*) venom in rats. *Toxicon* 1995;**33**:1491-9.

27. Bhattacharya SK, Mohan Rao PJR, Sen AP. Anxiogenic activity of intraventricularly administered bradykinin in rats. *J Psychopharmacol* 1995;**9**:348-54.

28. Bhattacharya SK, Bhattacharya A, Chakrabarti A. Anxiogenic action of intraventricularly administered arginine-vasopressin in the rat. *Biogenic Amines* 1998;**14**:367-85.

29. Bhattacharya SK, Bhattacharya A, Ghosal S. Anxiogenic activity of methylenedioxymethamphetamine (Ecstasy): an experimental study. *Biogenic Amines* 1998;**14**:217-37.

30. Bhattacharya SK, Chakrabarti A, Sandler M, Glover V. Rat brain MAO-A and MAO-B inhibitory (tribulin) activity during drug withdrawal anxiety. *Neurosci Lett* 1995;**199**:103-5.

31. Palit G, Kumar R, Patnaik GK, Bhattacharya SK. Behavioural effects of isatin, a putative biological factor, in rhesus monkeys. *Biogenic Amines* 1997;**13**:131-42.

32. Bhattacharya SK, Chakrabarti A, Sandler M, Glover V. Anxiolytic activity of intraventricularly administered atrial natriuretic peptide in the rat. *Neuropsychopharmacol* 1996; **15**:199-206.

33. Bhattacharya SK. Anticonvulsant activity of intraventricularly administered atrial natriuretic peptide and its inhibition by isatin. *Biogenic Amines* 1998;**14**:131-42.

34. Bhattacharya SK, Chakrabarti A, Sandler M, Glover V. Isatin inhibits the memory facilitating effect of centrally administered atrial natriuretic peptide in the rats. *Med Sci Res* 1996;**24**:299-301.

35. Satyan KS, Rai A, Jaiswal AK, Acharya SB, Bhattacharya SK. Isatin, a putative anxiogenic endocoid, induces memory dysfunction in rats. *Indian J Exp Biol* 1995;**33**:576-9.

36. Ghosal S, Bhattacharya SK, Muruganandam AV, Satyan KS. Iron - ion catalyzed oxidation of tryptophan - a simulated biosynthetic route to isatin. *Biogenic Amines* 1997;**13**: 91-100.

37. Bhattacharya SK, Chakrabarti A. Dose-related proconvulsant and anticonvulsant activity of isatin, a putative biological factor, in rats. *Indian J Exp Biol* 1998;**36**:118-21.

38. Bhattacharya SK, Acharya SB. Anxiogenic agents and 5-HT_3 receptors. In: Recent Advances in the study of neurotransmitter receptors. Dhawan BN, Srimal RC, Rapaka RS, Raghubir R, editors, CDRI, Lucknow, 1994:70-80.

39. Kulkarni SK, Reddy DS. Neurosteroids: a new class of neuromodulators. *Drugs of Today* 1995;**31**:433-55.

40. Reddy DS, Kulkarni SK. Role of $GABA_A$ and mitochondrial diazepam binding inhibitor receptor in the ant-stress activity of neurosteroids in mice. *Psychopharmacol* 1996; **126**:180-92.

41. Reddy DS, Kulkarni SK. Neuroprotective effects of neurosteroids against hypoxic stress-induced neurotoxicity in naive and FG 7142-treated mice. *Indian J Pharmacol* 1997;**29**:381-92.

42. Reddy DS, Kulkarni SK. Neurosteroid co-administration

prevent development of tolerance and augments recovery from benzodiazepine withdrawal anxiety and hyperactivity in mice. *Meth Find Exp Clin Pharmacol* 1997;**19**:395-405.

43. Reddy DS, Kulkarni SK. Chronic neurosteroid treatment prevents developments of morphine tolerance and attenuates abstenance behaviour in mice. *Eur J Pharmacol* 1997;**337**:19-25.
44. Reddy DS, Kulkarni SK. Potential mechanisms of neurosteroid -induced attenuation of morphine tolerance and dependence. *Indian J Pharmacol* 1998;**30**:6-15.
45. Reddy DS, Kulkarni SK. Differential anxiolytic effects of neurosteroids in the mirrored chamber behaviour test in mice. *Brain Research* 1997;**752**:61-71.
46. Reddy DS, Kulkarni SK. Reversal of benzodiazepine inverse agonist FG 7142 induced anxiety syndrome by neurosteroids in mice. *Meth Find Exp Clin Pharmacol* 1997;**19**:665-81.
47. Reddy DS, Kulkarni SK. Role of $GABA_A$ and mitochondrial diazepam binding inhibits receptors on the effects of neurosteroids on food intake in mice. *Psychopharmacol* 1998;**137**:391-400.
48. Reddy DS, Kulkarni SK. Sex and oestrus cycle-dependent in neurosteroid and benzodiazepine effects on food consumption and plus-maze learning behaviours in rats. *Pharmacol Behav* 1998;**62**:53-60.
49. Reddy DS, Kulkarni SK. The effects of neurosteroids on acquisition and retention of a modified passive avoidance learning task in mice. *Brain Res* 1998;**791**:108-16.
50. Reddy DS, Kulkarni SK. Possible role of nitric oxide in the nootropic and anti-amnesic effects of neurosteroids on aging- and dizocilpine -induced learning impairment. *Brain Res* 1998;**799**:215-29.
51. Reddy DS, Kulkarni SK. Inhibition of neuronal nitric oxide synthase (n-cNOS) reverses the corticotropin induced behavioural effects in rats. *Mol Cell Biochem* 1998;**183**:25-58.
52. Reddy DS, Kulkarni SK. Proconvulsant effects of neurosteroids pregnenolone sulfate and dehydroepiandrosterone sulfate in mice. *Eur J Pharmacol* 1998;**345**:55-59.
53. Reddy DS, Gurpreet Kaur, Kulkarni SK. Sigma (σ1) receptor mediated anti-depressant -like effects of neurosteroids in the Porsolt forced swim test. *Neuroreport* 1998;**9**:3069-73.
54. Kulkarni SK, Shaji AV. Behavioural pharmacology of melatonin. *Drugs of Today* 1998;**34**:237-47.
55. Shaji AV, Kulkarni SK. Evidence for GABA-ergic modulation of melatonin induced short-term memory deficits and food consumption. *Meth Find Exp Clin Pharmacol* 1998;**20**:311-9.
56. Shaji AV, Kulkarni SK. Central nervous depressant activities of melatonin in rats and mice. *Indian J Exp Biol* 1998; **36**:257-63.
57. Shaji AV, Kulkarni SK, Agrawala JN. Regulation of secretion of Il-4 and IgGl-isotope by melatonin stimulated ovalbumin specific T cells. *Clin Exp Immunol* 1998;**111**:181-5.
58. Ragavendra V, Chopra K, Kulkarni SK. Involvement of cholinergic system in losartan-induced facilitation of spatial and short term working memory. *Neuropeptides* 1998;**32**:417-21.
59. Ragavendra V, Chopra K, Kulkarni SK. Modulations of motor functions involving the dopaminergic system by AT1 receptor antagonist, losartan. *Neuropeptides* 1998;**32**:275-80.
60. Ramanathan M, Jaiswal AK, Bhattacharya SK. Differential effects of diazepam on anxiety in streptozotocin induced diabetes and non-diabetic rats. *Psychopharmacol* 1998; **135**:361-7.
61. Singh Y, Jaiswal AK, Singh M, Bhattacharya SK. Behavioural effect of prenatal diazepam administration on anxiety pattern in rats. *Indian J Exp Biol* 1996;**34**:1095-9.
62. Bhattacharya SK, Chakrabarti A. Vasopressin and atrial natriuretic peptide: from water homeostasis to behaviour. *Annals Neurosci* 1997;**6**:5-10.
63. Bhattacharya SK, Chakrabarti A, Glover V. Stress and water balance: the roles of ANP, AVP and isatin. *Indian J Exp Biol* 1998;**36**:1195-200.
64. Palit G, Kumar R, Chowdhury SR, Gupta MB, Saxena RC, *et al.* A primate model of anxiety. *Eur J Neuropsychopharmacol* 1998;**8**:195-201.
65. Palit G, Chowdhury SR. Gupta MB, Dhawan KN, Srimal RC, Dhawan BN. Model of anxiety in primates. *Can J Physiol Pharmacol* 1994;**72**:450.
66. Prathiba J, Kumar KB, Karanth KS. Fear -potentiated poststartle activity in neonatal clomipramine treated rats. *Indian J Pharmacol* 1997;**20**:201-3.
67. Kumar KB, Karanth KS. Effect of DSP-4 induced depletion of brain norepinephrine on appetitive and aversive memory retrieval. *Indian J Exp Biol* 1994;**32**:724-8.
68. Singh HK, Srimal RC, Dhawan BN, Involvement of muscarnic receptor in the retrieval of shock - motivated brightness discrimination response. In: Dhawan BN, Srimal RC, Rapaka RS, Raghubir R, Editors. Recent advances in the study of neurotransmitter receptors, CDRI, Lucknow, 1994:213-8.
69. Andrade C, Rao KS, Sekhar RV, Nagarani MA, Venkataraman BV. Relationship between rate of administration of ECS- and rate of learning in rats: implications for the practice of ECT. *Convulsive Therapy* 1994;**10**:206-11.

70. Andrade C, Joseph J, Chandra S, Venkataraman BV, Nagarani MA. ECT-induced antrograde amnesia: can the deficits be minimized. *Convulsive Therapy*,1994;**10**:59-64.

71. Kamath S, Andrade C, Faruqui S, Venkataraman BV, Nagarani MA, Candade ME. Evaluation of pre ECS-antihypertensive drug administration in the attenuation of ECS-induced retrograde amnesia. .*Convulsive Therapy*.\ 1997;**13**:185-95.

72. Joseph J, Venkataraman BV, Nagarani MA, Andrade C. BR-16A protects against ECS-induced antrograde amnesia. *Biol Psychiatry* 1994;**36**:478-81.

73. Ramteke S, Andrade C, Faruqui S, Joseph J, Venkataraman BV, Nagarani MA. BR-16A attenuates antrograde amnesia induced by electroconvulsive shock in slow learning in rats. *Indian J Pharmacol* 1995;**27**:186-8.

74. Sudha S, Lakshmana, MK, Pradhan N. Changes in learning and memory, acetyl cholinesterase activity and monoamines in the brain after chronic carbamazepine administration in rats. *Epilepsia* 1995;**36**:416-22.

75. Kumar KB, Karanth KS. Effects of ACTH and ACTH 4-10 on aversive memory retrieval in rats. *J Neural Trans* 1995;**101**:223-9.

76. Kumar KB, Karanth KS. Effect of central administration of arginine -vasopressin on aversive memory retrieval. *Brain Res* 1995;**699**:293-6.

77. Kumar KB, Karanth KS. Alpha-helical CRF blocks differential influence of corticotropin releasing factor (CRF) on appetitive and aversive memory retrieval in rats. *J Neural Trans* 1996;**103**:1117-26.

78. Kumar KB, Karanth KS. Effect of central administration of oxytocin on appetitive and aversive memory retrieval. *Indian J Clin Psychol* 1997;**24**:113-6.

79. Kumar KB, Nalini K, Karanth KS. Effect of p-chlorophenyl alanine-induced depletion of brain serotonin on retrieval of appetitive and aversive memories. *Indian J Exp Biol* 1995;**33**:837-40.

80. Goswami M, Mund S, Ray A. Effect of some psychotropic agents on cognitive functions in rats. *Indian J Physiol Pharmacol* 1996;**40**:75-8.

81. Kumar KB, Karanth KS. Scopalamine blocks the effects of swim stress on memory retrieval in rats. *J Neural Trans* 1996;**103**:1331-6.

82. Sudha S, Lakshmana MK, Pradhan N. Chronic phenytoin induces impairment of learning and memory with associated changes in brain acetyl choline esterase activity and monoamine levels. *Pharmacol Biochem Behav* 1995; **52**:119-24.

83. Kumar KB, Ramalingam S, Karanth KS. Phenytoin and phenobarbital: a comparison of their state dependent effects. *Pharmacol Biochem Behav* 1994;**47**:951-6.

84. Vohora D, Pillai KK. Proconvulsant effect of buspirone and its modulation by α_2 adrenoreceptor agonist in mice. *Indian J Pharmacol* 1998;**30**:329-33.

85. Joseph S, David J, Joseph T. Determination of anticonvulsant effects on seizure thresholds using ramp generated cortical stimulation in young rats. *Indian J Exp Biol* 1997;**35**:933-40.

86. Kulkarni C, David J, Joseph T. Influence of adenosine, dipyridamol, adenosine antagonists and antiepileptic drugs on EEG after discharge following cortical stimulation. *Indian J Exp Biol* 1997;**35**:342-7.

87. Kulkarni SK, George B. Kindling model of epilepsy. *Meth Find Exp Clin Pharmacol* 1994;**16**:735-45.

88. Kulkarni SK, George B. Lithium-pilocarpine neurotoxicitry: a potential model of status epilepticus. *Meth Find Exp Clin Pharmacol* 1995;**17**: 551-7.

89. George B, Kulkarni SK. Protective effects of GABA-ergic drugs and other anti-convulsive in lithium-pilocarpine -induced status epilepticus. *Meth Find Exp Clin Pharmacol* 1996;**18**:335-40.

90. George B, Kulkarni SK. Modulation of lithium-pilocarpine - induced status epilepticus by adenosinergic agents. *Meth Find Exp Clin Pharmacol* 1997;**19**:329-33.

91. George B, Kulkarni SK. Protective effect of clozapine against pentylenetetrazol convulsions and kindling. *Meth Find Exp Clin Pharmacol* 1998;**20**;395-401.

92. George B, Mathur R, Kulkarni SK. Development of self sustaining limbic status epilepticus by continuous ventral hippocampal stimulation followed by low dose pilocarpine in rats. *Indian J Physiol and Pharmacol* 1998;**42**:359-68.

93. Kulkarni SK, Ninan I. Current concepts in molecular diversity and pharmacology of dopamine receptors. *Meth Find Exp Clin Pharmacol* 1996;**18**:599-613.

94. Ninan I, Kulkarni SK. Partial agonistic action of clozapine at dopamine D_2 receptors in dopamine depleted animals. *Psychopharmacol* 1998;**135**:311-7.

95. Ninan I, Kulkarni SK. Clozapine: partial; agonist at dopamine D_2 receptors ! Psychopharmacol 1998;**138**:215-6.

96. Ninan I, Kulkarni SK. Preferential blockade by clozapine of hyperlocomotion induced by non-competitive NMDA-antagonist MK-801. *Indian J Physiol Pharmacol* 1998;**42**:375-82.

97. Ninan I, Kulkarni SK. 5-HT_{2A} receptor antagonists block MK-801-induced stereotypy and hyperlocomotion. *Eur J Pharmacol* 1998;**358**;111-6.

98. Ninan I, Kulkarni SK. Dopamine receptor sensitive effect of dizocilpine on feeding behaviour. *Brain Res* 1998; **812**:157-63.

99. Ninan I, Kulkarni SK. Clozapine-induced cognitive dysfunction's in mice. *Meth Find Exp Clin Pharmacol* 1996; **18**:367-72.

100. Ninan I, Kulkarni SK. Antimuscarnic effects of clozapine in rats. *Pharmaceutical Sci* 1996;**2**:427-8.

101. Rauniar GP, Gitanjali B, Shashindran CH. Comparative effects of hyoscine butyl bromide and atropine sulphate on sleep architecture in healthy human volunteers. *Indian J Physiol Pharmacol* 1998;**42**:395-400.

102. Gitanjali B, Rauniar GP, Shashindran CH. Effect of hyoscine butyl bromide and atropine on heart rate during nocturnal sleep. *Indian J Exp Biol* 1998;**36**:1216-20.

103. Gitanjali B. Effect of the karnatic music raga "Neelambari" on sleep architecture. *Indian J Physiol Pharmacol* 1998; **42**:119-22.

104. Gitanjali B. Establishing a polysomnography laboratry in India: problems and pitfalls. *Sleep* 1998;**21**:331-32.

105. Gitanjali B, Shashindran CH. Sleep medicine-an emerging medical specialty. *J Applied Med* 1997;809.

106. Nath C, Patnaik GK, Haq N, Mathur KB, Srimal RC, Dhawan BN, Brreca F. Novel met-enkephalin analogue; a potent systemic Mu agonist antinociceptive agent. *Pharmacol Res* 1995;**31**:269-73.

107. Katti SD, Misra PK, HaqW, Mathur KB, Raghubir R, Patnaik GK, Dhawan BN. Synthesis and analgesic activity of dermorphin analogues. *Indian J Chem* 1994; **33**:445-50.

108. NaqviT, Haq W, Raghubir R, Tripathi A, Patnaik GK, Mathur KB. Synthesis and opioid activity of novel tetrapeptides analoges to sequence (1-4) of dermorphin. *Neuropeptides* 1998;**32**:333-37.

109. Raghubir R, Naqvi T, Varma S, Haq W, Mathur KB, Patnaik GK. Synthesis and opioid activity of novel dermorphin related heptapeptides. *Med Chem Res* 1997; **7(1)**:16-24.

110. Sondhi SM, Singhal N, Verma RP, Raghubir R, Goel AK, Patnaik GK. Synthesis anti-inflammatory and analgesic activity evaluation of some 2-(9-acridinylamino) pyridines and (2-9-acridinylamine/imino) thiazolines. *Indian J Chem* 1997;**36B**:620-24.

111. Raghubir R, Kumar A, Dhawan BN. Nature of Neurotransmitters involved in pretectal stimulation produced analgesia. In : Recent Advances in the Study of Neurotransmitter Receptors, CDRI, Lucknow 1994; 282-88.

112. Kumar A, Raghubir R, Dhawan BN. Antinociception induced by neurotransmitter agonists microinjected into the pretectal nucleus of rat. In : Recent Advances in the Study of Neurotransmitter Receptors, CDRI, Lucknow 1994; 255-81.

113. Kumar A, Raghubir R, Dhawan BN. Possible involvement of nitric oxide in red nucleus stimulation-induced analgesia in the rat. *Eur J Pharmacol* 1995; **279**:1-5.

114. Dhawan BN, Palit G, Raghubir R. Neurohumoral modulation of analgesia. In : Pain Mechanism and Management. INSA Diamond Jubilee Publication. Ed. KN Sharma, INSA, New Delhi 1995; 73-90.

115. Hegde LG, Shukla R, Dikshit M, Srimal RC. The role of arginine EDRF/NO pathway in cardiovascular regulation of cat. *Indian J Pharmacol* 1994;**26**:66-67.

116. Shukla A, Dikshit M, Srimal RC. Regulation of blood brain barrier by nitric oxide. *Can J Physiol Pharmacol* 1994; **72**:474.

117. Shukla A, Dikshit M, Srimal RC. Nitric oxide modulates blood-brain barrier permeability during infections with an inactivated bacterium. *Neuroreport* 1995;**6**:1629-32.

118. Shukla A, Dikshit M, Srimal RC. Nitric oxide -dependent blood-brain barrier permeability alteration in the rat brain. *Experimentia* 1996;**52**:134-6.

119. Agrawal A, Shukla R, Tripathi LM, Pandey VC, Srimal RC. Permeability function in relation to cerebral microvessel enzymes during aging in rats. *Int J Devl Neurosci* 1996; **14**:87-91.

120. Hegde LG, Dikshit M, Shukla R, Srimal R. Interrelationship between blood pressure level and brain nitric oxide synthase (NOS) activity in rats. *J Neurochem* 1994;**63**:11.

121. Shukla R, Rahmani NH, Mizuma H, Srimal RC, Prasad C. Role of endogenous cyclo (His-Pro) in cold induced hypothermia in desert rat (Mastomys natalensis). *Peptides* 1994;**15**:1471-4.

122. Shukla A, Srimal RC, Prasad C. Cyclo (His-Pro) modulation of body temperature at hot ambient temperature in the desert rat (Mastomys natalensis). *Peptides* 1997; **18**:689-93.

123. Carlton J, Khan SI, Haq W, Mizuma H, Ragan FA *et al.* Attenuation of alcohol induced hypothermia by cyclo (His-Pro) and its analogs. *Neuropeptide* 1995;**28**:351-5.

124. Nargund LVG, Reddy YSR, Samevenkatesh, Mishra U, Suresh B. Effect of 2-N (substituted) amino-pyrido [1,2-9] pyrimidin-4-oxo-1H-3-carbonitriles on the analgesic activity in albino mice. *Indian drugs* 1995;**32**:458-60.

125. Ramasarma GVS, Azam MDA, Suresh B. Synthesis and biological activities of 2-(N-substituted aminoethyl)-1,3-diphenyl-3-(4-methoxyphenyl)-propane 1,3-diones. *Indian drugs* 1996;**36**:267-71.

126. Subramani P, Suresh B, Ramasarma GVS. N-(4-benzylidine-2-methyl/phenyl-5-oxo-imidazolin-1-yl)-acetamides and their-(- (4-substituted) azo analogs. *Indian J Hetrocyclic Chem* 1997;**6**:215-8.

127. Bhattacharya SK, Chakrabarti A, Chatterjee SS. Activity profile of two hyperforin - containing Hypericum extracts in behavioural models. *Pharmacopsychiat* 1998;**31**:22-9.

128. Chatterjee SS, Bhattacharya SK, Wonnemann M, Singer A, Muller WE. Hyperforin as a possible antidepressant component of hypericum extracts. *Life Sci* 1998;**63**:499-510.

129. Bhattacharya SK, Ghosal S. Anxiolytic activity of standardised extracts of *Bacopa monniera*: an experimental study. *Phytomedicine* 1998;**5**:77-82.

130. Jaiswal AK, Bhattacharya SK, Acharya SB. Anxiolytic activity of Azadirachta indica leaf extract in rats. *Indian J Exp Biol* 1994;**32**:489-91.

131. Ghosal S, Muruganandam AV, Singh SK, Satyan KS, Bhattacharya SK *et al.* The chemistry and actions of 6-alkylsalicylates of Indian *Ginkgo biloba. Indian J Chem* 1997;**36**:257-63.

132. Satyan KS, Jaiswal AK, Ghosal S, Bhattacharya SK. Anxiolytic activity of ginkgolic acid conjugates from Indian Ginkgo biloba. *Psychopharmacol* 1998;**136**:148-152.

133. Bhattacharya SK, Kumar A, Ghosal S. Effects of glycowithanolides from *Withania somnifera* on an animal model of Alzheimer's disease and perturbed cholinergic markers of cognition in rats. *Phytother Res* 1995;**9**:110-13.

134. Bhattacharya SK. Effect of Trasina®, an Ayurvedic herbal formulation, on experimental models of Alzheimer's disease and central cholinergic markers in rats. *J Altern Complement Med* 1998;**3**:327-67.

135. Bhattacharya SK, Kumar A, Jaiswal AK. Effect of Mentat, a herbal formulation, on experimental models of Alzheimer's disease and central cholinergic markers in rats. *Fitoterapia* 1995;**56**:216-22.

136. Iyer MR, Pal SC, Kasture US, Kasture SB. Effect of *Lawsonia inermis* on memory and behaviour mediated via neurotransmitters. *Indian J Pharmacol* 1998;**30**:181-5.

137. Bhattacharya SK, Satyan KS, Ghosal S. Antioxidant activity of glycowithanolides from *Withania somnifera. Indian J Exp Biol* 1997;**35**:236-9.

138. Bhattacharya SK, Bhattacharya A, Ghosal S. Antioxidant activity of Ocimum sanctum: an experimental study. *Indian J Med Biochem* 1997;**1**:12-8.

139. Ghosal S, Bhattacharya SK. Antioxidant defense by native and processed Shilajit : a comparative study. *Indian J Chem* 1996; **35B**:127-32.

140. Bhattacharya SK, Sen AP, Ghosal S. Effects of Shilajit on biogenic free radicals. *Phytother Res* 1995;**9**:56-9.

141. Bhattacharya SK, Ghosal S. Experimental evaluation of the antistress activity of a herbal formulation Zeetress. *Indian J Indg Med* 1994;**10**:1-8.

142. Ahmad G, Amin KMY, Khan NA, Tajuddin. The anti-stress activity of gem-containing Unani formulation against diverse stressors. *J Ethnopharmacol* 1998;**59**:187-93.

143. Mitra SK, Chakrabarti A, Bhattacharya SK. Neuropharmacological studies on *Panex ginseng. Indian J Exp Biol* 1996;**34**:41-7.

144. Mazumder UK, Gupta M, Rath N. CNS activity of Cassia fistula in mice. *Phytother Res* 1998;**12**:520-2.

145. Gupta M, Mazumder UK, Bhawal SR, Swamy SMK. CNS activity of petroleum ether extract of *Vitex negundo* Linn in mice. *Indian J Pharm Sci* 1997;**59**:240-45.

146. Vidya PM, Karanth KS, Rao A. Effect of Nardostachys jatamansi on biogenic amines and inhibitory amino acids in rat brain. *Planta Med* 1994;**60**:114-17.

147. Bajaj S, Vohora SB. Analgesic activity of gold preparation used in Ayurveda and Unani-TIBB. *Indian J Med Res* 1998; 104-11.

148. Bajaj S, Vohora SB. Gold preparations used in Ayurveda and Unani-TIBB, therapeutic rationale and safety aspect. *Abstract presented in ISTERH appeared in the J Trace Elements in Experimental Medicine* 1998;**59**:187-93.

149. Vohora SB, Khanna T, Athar M, Ahmad B. Analgesic activity of Bacosine, a new triterpine isolated from *Bacopa monnieri. Fitoterapia* 1997;**68**:361-5.

150. Khanna N, Goswami M, Sen P, Ray A. Antinociceptive action of *Azadirachta indica* (neem) in mice: possible mechanisms involved. *Indian J Exp Biol* 1995;**33**:848-50.

151. Vohara SB. Preliminary behavioural and neurological studies on Kushta Marjan and Kushta Khar Mohara used in Unani-TIBB. *Aryavaidyan* 1997;**10**:67-73.

152. Gupta M, Mazumder KK, Chakrabarti S, Bhattacharya S, Rath N, Bhowal SR. Anti-epileptic and anti-cancer activity of some indigenous plants. *Indian J Phiysiol Allied Sci* 1997;**51**:53-6.

153. Kulkarni SK, George B, Nayar U. Amygdaloid kindling in rats: protective effect of *W.somnifera* root extract. *Indian drugs* 1995;**32**:37-49.

154. Kulkarni SK, George B. Anticonvulsant action of W. somnifera root extract against PTZ-induced kindling in mice. *Phytotherapy Res* 1995;**9**:447-49.

155. Kulkarni SK, George B, Mathur R. Neuroprotection by *Withania somnifera* root extract against lithium-pilocarpine -induced seizures. *Indian drugs* 1998;**35**:208-15.

156. Kulkarni SK, George B, Mathur R. Protective effect of *Withania somnifera* root extract on electrographic activity in lithium-pilocarpine model of status epilepticus. *Phytotherapy Res* 1998;**12**:451-3.

157. Kulkarni SK, George B. Pentylenetetrazol -induced kindling in animals: protective effect of BR-16A. *Indian J Exp Biol* 1995;**33**:424-7.

158. Kulkarni SK, Joseph P. Anticonvulsant profile of Siotone granules®, a herbal preparation. *Indian J Exp Biol* 1998; **36**:658-62.

159. Kulkarni SK, Sharma A. reversal of diazepam induced withdrawal hyperactivity in mice by BR-16A (Mentat®), a herbal preparation. *Indian J Exp Biol* 1994;**32**:886-8.

160. Kulkarni SK, Sharma DK, George B. Triazolam withdrawal-induced hyperactivity and anxiety in mice: prevention by BR-16A, a herbal psychotropic formulation. *Indian Drugs* 1997;**34**:203-7.

161. Kulkarni SK, Ninan I. Inhibition of tolerance and dependence to opiate by *Withania somnifera* in mice. *J Ethanopharmacol* 1997;**57**:213-7.

162. Kulkarni SK, Joseph P. Psychopharmacological profile of Siotone granules, a herbal psychotropic preparation. *Indian drugs* 1998;**35**:536-54.

163. Gurpreet Kaur, Kulkarni SK. Reversal of forced swimming -induced chronic fatigue in mice by antidepressant and herbal psychotropic drugs. *Indian drugs* 1998;**35**:771-7.

164. Kulkarni SK, Goel RK. Gastric antiulcer activity of UL-409 in rats. *Indian J Exp Biol* 1998;**34**:683-8.

165. Kulkarni SK, Ninan I. Spirulina-induced hyperlocomotor activity and stereotypy in mice. *Indian drugs* 1996;**33**:517-20.

166. Schlioebs R, Liebmann A, Bhattacharya SK, Kumar A, Ghosal S, Bigl V. Systemic administration of defined extracts from *Withania somnifera* (Indian Ginseng) and shilajit differentially affects cholinergic but not glutaminergic and gabaergic markers in rat brain. *Neurochem Int* 1996;**30**:181-90.

167. Satyan KS, Jaiswal AK, Ghosal S, Bhattacharya SK. Effects of ginkgolic acid conjugates on the brain monoamines and metabolites in rodents. *Biogenic Amines* 1997;**13**:143-51.

168. Muruganandam AV, Jaiswal AK, Ghosal S, Bhattacharya SK. Effect of *Wrightia tinctoria* on the brain monoamines and metabolites in rats. *Biogenic Amines* 1998;**14**:655-65.

169. Ganguly S, Hota D, Goel RK, Acharya SB, Bhattacharya SK. Neuropharmacological studies on Fusarium toxins, I. Total toxin extract from *F. moniliforme*. *Indian J Exp Biol* 1996;**34**:408-17.

170. Ganguly S, Hota D, Goel RK, Acharya SB, Bhattacharya SK. Neuropharmacological studies on Fusarium toxins, II. Total toxin extract from *F. oxysporum*. *Indian J Exp Biol* 1996;**34**:418-26.

171. Ganguly S, Hota D, Goel RK, Acharya SB, Bhattacharya SK. Neuropharmacological studies on Fusarium toxins, III. Neurochemical investigations on total toxin extract from *F. moniliforme* and *F. oxysporum*. *Indian J Exp Biol* 1996;**34**: 531-4.

172. Husain R, Agrawal AK, Husain M, Seth PK. Modulation of acrylamide-induced neurochemical and behavioural deficits by cerebellar transplantation in rats. *Pharmacol Biochem Behav* 1994;**49**:443-8.

173. Khanna VK, Husain R, Seth PK. Effect of protein malnutrition on the neurobehavioural toxicity of styrene in young rats. *J Applied Toxicol* 1994;**14**:351-6.

174. Agrawal AK, Husain R, Raghubir R, Kumar A, Seth PK. Neurobehavioural, neurochemical and electrophysiological studies in 6-hydroxydopamine lesional and neural transplanted rats. *Int J Dev Neurosci* 1995;13:105-11.

175. Adhami VM, Husain R, Husain R, Seth PK. Influence of iron deficiency and lead treatment on behaviour and cerebellar and hipocampal polyamine levels in neonatal rats. *Neurochemical Res* 1996;**21**:921-8.

176. Shukla A, Shukla GS, Srimal RC. Cadmium induced alterations in blood-brain barrier permeability and its possible correlation with microvessal antioxidant potential in rat. *Human Exper Toxicol* 1996;**15**:400-4.

177. Roy A, Agrawal AK, Husain R, Seth PK. Restoration of trimethyltin induced hippocampal degeneration of single and multiple transplantation. *Restorative Neurol Neurosci* 1997;**11**:195-201.

178. Roy A, Agrawal AK, Husain R, Dubey MP, Seth PK. Cholinergic and serotonergic alterations in the rat hippocampus following tirmethyltin exposure and fetal neural transplantation. *Neurosci Lett* 1998;**259**:1-4.

179. Husain R, Husain R, Adhani VM, Seth PK. Behavioural, neurochemical and neuromorphological effects of deltamethrin in adult rats. *J Toxicol Environ Health* 1996; **B48**:101-12.

180. Vohora SB, Kim HS, Ganotra KB, Mishra GV, Bajaj AS *et al.* Use of toxic metals in Indian system of medicine: Therapeutic rationale and safety aspects. *Ethnotherapies* 1998;**14**:101-4.

181. Agarwal AK, Husain R, Raghubir R, Kumar A, Seth PK. Neurobehavioural neurochemical and electrophysiological studies in 6-hydroxydopamine lesioned and neural transplanted rats. *Int J Dev Neuroscience* 1995;**13**:105-11.

182. Varma S, Raghubir R, Patnaik GK. Nitric oxide modulation of cardiovascular responses from ventral surface of medulla in cat. *Indian J Pharmacol* 1997;**29**:125-8.

183. Srivastava MP, Makde SD, Paranjpe BD. Interaction of ciprofloxacin with diclofenac and paracetamol in relation to its epileptogenic effect. *Indian J Physiol Pharmacol* 1997;**41**:164-6.

184. Joseph S, David J, Joseph J. Synergistic effect of flunarizine and sodium valproate on seizure threshold elicited by cortical stimulation in conscious rats. *Indian J Physiol Pharmacol* 1998;**42**:39-49.

185. Joseph S, David J, Joseph J. Additive anticonvulsant effect of flunarizine and sodium valproate on electroshock and chemoshock induced seizures in mice. *Indian J Physiol Pharmacol* 1998;**42**:383-8.

186. Singh KP, Jaiswal AK, Singh M, Bhattacharya SK. Behavioural alterations in rats induced by single prenatal exposure of haloperidol. *Indian J Exp Biol* 1998;**36**:1102-07.

187. Singh Y, Jaiswal AK, Singh M, Bhattacharya SK. Effect of prenatal haloperidol administration on anxiety patterns in rats. *Indian J Exp Biol* 1997;**35**:565-75.

188. Jaiswal AK, Bhattacharya SK. Effect of prenatal undernutrition and phenobarbitone administration on discrimination learning and passive avoidance behaviour in rats. *Indian J Exp Biol* 1996;**34**:118-23.

189. Bhattacharya SK, Satyan KS,. Experimental methods for the evaluation of psychotropic drugs in rats. antianxiety agents. *Indian J Exp Biol* 1997;**35**:565-75.

190. Bhattacharya SK, Satyan KS, Ramanathan M. Experimental methods for evaluation of psychotropic agents: II Antidepressants. *Indian J Exp Biol* 1997;**37**:117-23.

191. Chakrabarti A, Bhattacharya SK. New targets for novel analgesics. *Indian J Pain* 1996;**10**:3-9.

192. Chakrabarti A, Tandon R, Bhattacharya SK. Analgesics: new vistas. *J Int Med* 1997;**8**:22-6.

193. Bhattacharya SK. Rationale management of postoperative pain in children. In: Pain. Rudra A, Ray M, Tripathi SK.(eds) Indian Soc for Study of Pain , Calcutta, 1997, 41-9.

194. Chakrabarti A, Ganguly AN, Bhattacharya SK. Rationale use of analgesic for acute and post operative pain in children. *Quart J Surg Sci* 1996 37-44.

195. Chakrabarti A, Biswas R, Tandon R, Bhattacharya SK. Recent approaches to pharmacological management of senile dementia. *J Int Med* 1996;**7**:18-21.

196. Chakrabarti A, Bhattacharya SK. Selective serotonin reuptake inhibitors: clinical use in psychiatry. *J Int Med* 1995; **11**:88-91.

197. Chakrabarti A, Bhattacharya SK. Sumitriptan: a new drug for migraine and cluster headache. *Indian J Clin Pract* 1996;**7**:39-42.

198. Srimal RC. The blood-brain barrier: some recent developments. *Ann Natl Acad Sci (India)* 1997;**33**:209-22.

TOXICOLOGY

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World over, the discipline of toxicology is rapidly advancing with multidisciplinary inputs. Both regulatory and investigative toxicology are being pursued in R & D laboratories, medical colleges and drug houses in India. Regulatory toxicology is being predominantly pursued in drug houses. Adequate facilities for regulatory toxicology are available in the country and investigators are aware of Good Laboratory Practices (GLP) and observe them. Under investigative toxicology, current stress is on understanding the toxic effects of chemicals at molecular level to predict the toxicogenic potential of chemicals and identify the specific targets of toxicity. A significant amount of work is being undertaken on monitoring of the toxicants such as pesticides, metals and other chemicals in water and food materials and also non-permitted colours in the latter. The monitoring studies have not been included in this report due to the constraint of space and being not directly related with the subject.

1. Pesticides

Reports of the presence of pesticides in water and food materials have aroused great concern among the health scientists and regulatory agencies and hence led to studies on their toxic potential. Several groups are working on the mechanism of toxicity of pesticides.

1.1. Organochlorines

Involvement of cellular calcium homeostatic mechanisms in the cardiovascular toxicity of gamma-hexachlorocyclohexane (gamma-HCH) has been reported[1]. An evaluation of the age factor on the accumulatory pattern of different HCH isomers and their tissue specific distribution studied in rats might be extrapolated to human exposure[2]. Changes in haemoglobin and electrocardiograms were shown to be the early effects of HCH exposure among farm workers[3]. In a study on HCH exposure in rat erythrocyte membranes and brain, a drastic reduction in phosphoinositide levels is reported suggesting adverse effects on vital membranes and cell functions modulated by phosphoinositides[4].

A significant increase in lipid peroxides and total glutathione content in liver, 6 hrs. after HCH treatment which remained higher till 24 hrs. was reported in a study on hepatic oxidative stress parameters in chicks[5]. Protein content of crude homogenate and 10,000 g supernatant were found to be decreased after 6hrs. Further studies on immature (7 day old) and young (30 day old) chicks revealed that lipid peroxidation (LPO) increased only in nuclear fraction in the latter while in the immature chicks LPO increased in the subcellular fractions after HCH exposure. The study also reported that LPO following treatment of liver subcellular fractions with HCH *in vitro* is high in young chicks in comparison to immature ones. Cytoplasmic CN^- sensitive and resistant superoxide dismutase (SOD) activity were inhibited in immature chicks[6].

Significant quantities of HCH isomers were detected in testicular plasma membranes in rats following dermal exposure to HCH for 60 days along with decrease in the activity of 5'-nucleotidase, Ca^{2+}-ATPase, Na^+,K^+-ATPase and Mg^{2+}-ATPase, marker enzymes of testicular plasma membranes[7]. HCH was also reported to produce age dependent effects on testicular antioxidant defense enzymes and lipid peroxidation and involve oxidative stress in its toxicity in rats[8].

Testicular and sperm toxic effects of dermally applied HCH have also been reported[9].

Residual levels of technical HCH isomers in the intestinal epithelial cells were identified in rats following single oral exposure (50 mg/kg) after 1,2,3 and 5 days[10]. In an another study, influence of technical HCH and fasting-feeding was studied on rat intestinal epithelial cell lining[11]. Sialic acid content was found to be increased while hexose and sucrase levels decreased following HCH treatment during fasting.

HCH and its isomers were found to accumulate in different brain regions in a dose and time dependent manner in rats following dermal exposure[12]. Humoral immune suppression and increased levels of serum thiobarbituric acid reactive substance (TBARS) in rats following exposure to DDT (100 and 200 ppm) and lindane (40 and 80 ppm) and involvement of free radicals in organochlorine induced immunotoxicity have been reported[13]. The toxic effects of pesticides on these parameters were found to be attenuated following simultaneous treatment with ascorbic acid (100 mg/kg).

Influence of dietary protein and stress on DDT induced immune response was reported in rats and mice[14,15]. Study on the comparative evaluation of immunotoxicity of DDT and its metabolites was also carried out[16].

Exposure to endosulfan orally, either at a single dose (8 mg/kg) or at two doses (2 mg/kg) at weekly intervals, caused convulsions, muscular ataxia, hyperesthesia and excitement in male calves. All the animals died within three weeks and the study suggested that endosulfan has a great potential to produce toxic manifestations in young calves[17].

Sperm abnormalities and decreased sperm release in the epididymis along with a significant elevation of some testicular enzymes, was observed in animals treated with endosulfan[18].

In a study on rats, exposure to endosulfan (15 mg/kg) for 18 days caused a marked reduction in the levels of both phoshatidylinositol bis phosphate (PIP_2) and phosphatidylinositol(PI) and these effects were restored to near normal levels 2 weeks after cessation of treatment. However, single oral administration of endosulfan (15 mg/kg) reduced brain PI and PIP_2 without any effect on phoshatidylinositol 4-phosphate (PIP) levels in rats 4 hours after exposure suggesting possible involvement of phosphoinositide second messengers in the neurotoxicity of endosulfan[19].

Effect of pesticides on lymphocyte function has been reported suggesting that it could lead to pesticide induced immunosuppression[20].

Lindane suppressed the primary and secondary humoral immune responses in a time and dose dependent manner with a threshold susceptibility in mice[21].

1.2. Carbamates

Diuron, thiram and propoxur were found to significantly induce micronucleus formation in bone marrow cells and thiram induced a significant number of dead implants in male mice[22,23]. Diuron induced frequency of micronucleated erythrocytes was independent of the sex of the test animals[22]. Propoxur also induced micronucleus formation in bone marrow of Swiss mice. Pretreatment with indole-3-carbinol (500 mg/kg), a glucobrassicin derivative present in cruciferous vegetables, inhibited micronuclei formation[24].

Exposure to thiram caused time and dose dependent morphological and biochemical changes in testes[25]. Serum cholesterol and testicular free sialic acid were increased and testicular protein decreased in the treated rats.

A number of pesticides were found to affect testes in experimental studies. *In utero* or lactational exposure of carbofuran, a widely used pesticide, led to the production of poor quality sperm which may exert antifertility or male mediated developmental toxic effects[26]. Fungicide mancozeb induced biochemical and structural changes in gonads of male rats[27] and caused functional changes in the thyroid[28].

Decline in epididymal sperm count and sperm motility, an increase in sperm with abnormal morphology has been reported in male rats fed carbaryl for 60 days[29]. Suppression of cell mediated immune response has been reported in lambs fed carbofuran, for six months. A significant suppression in total leucocyte count, lymphocyte count and lymphocyte stimulation tests was observed and delayed type hypersensitivity of pesticide suggested[30].

1.3. Pyrethroids

Cypermethrin was found to suppress serum globulins, gamma globulins and specific haemagglutination-inhibition (HI) and ELISA antibodies in chickens[31].

Permethrin at higher dose (120 mg/kg/day) caused suppression of cell mediated immune response without any significant effect on humoral immune response in mice exposed for 14 days[32]. Permethrin was also found to cause chemical injury and interfere with carbohydrate and lipid metabolism in rats treated for 15 days at different doses (24-120 mg/kg). No apparent signs of toxicity were observed at lower doses (30-60 mg/kg/day) however, at higher doses (80 and 120 mg/kg), an increase in alanine aminotransferase (ALT), aspartate amino transferase (AST) and blood glucose levels was observed[33]. Deltamethrin in adult rats caused significant neurochemical and neuromorphological changes indicating alterations in synaptic function[34]. Brain polyamine levels were significantly altered in young rats exposed to deltamethrin and changes were region specific[35]. The study also reported significant alterations in brain dopamine and muscaranic receptors and associated behaviour.

Acute toxicity study of fluvalinate were carried out both in male and female rats at four different doses (62.5, 125, 250 and 500 mg/kg)[36]. The study showed that although the pesticide is moderately toxic, it has a potential to affect liver and kidney.

Fenvalerate when administered intraperitoneally in male rats for 45 days, induced hyperexcitability, tremors and paralysis and caused significant elevation of circulatory thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) and total calcium in brain[37].

Effect of testosterone propionate in fenvalerate-induced thyroid dysfunctions, as evident by a decrease in hepatic type I iodothyronine 5' monodeiodinase and serum triiodo thyronine (T_3) levels and lipid peroxidation was reported in mice[38].

1.4. Organophosphates

Phorate, a commonly used pesticide was reported to exert its neurotoxic effects by decreasing the activity of acetylcholinesterase and butyrylcholinesterase in olfactory bulb of albino mice[39].

Dimethoate was found to alter thyroid functions through the extrathyroidal conversion of T_4 to triidothyronine (T_3) and not through the hypophyseal thyroid axis. Possible involvement of lipoperoxidative process in the inhibition of hepatic type-I iodothyronine 5'-monondeiodinase (5'D) was suggestd by Maiti and Kar[40]. The pesticide was reported to cause high mortality in pregnant rats and pups born to the exposed animals exhibited a decrease in acetylcholinesterase activity in brain and placenta indicating migration of the pesticide to fetus[41]. Dimethoate impaired crosslinking of collagen and the effect was more in young rats compared to older ones[42]. Rats fed high protein diet were found to be resistant and showed protective action against the pesticide than those fed with normal diet. Acute toxic effects of malathion, carbaryl and cypermethrin were compared on testes in mice at different doses (1/20, 1/10 and 1/5 th of LD_{50}) after 24 hours and 7 days exposure. Cypermethrin produced significant abnormalities at 1/10th and 1/5th doses of LD_{50} in the sperm morphology in mice as compared to malathion and carbaryl[43]. Phosphamidon, a neurotoxic pesticide, was reported to affect testicular function with Leydig cells being the primary target[44].

Methyl parathion, Bayleton and Hinosan were found to be cytotoxic and produce cytogenic effects[45]. Dithane M-45 and Baygon (carbamates) were found to be non-genotoxic in albino mice[46].

Treatment with methyl parathion in hemicastrated virgin rats for 10 and 15 days resulted in an inhibition of ovarian compensatory hypertrophy and also significantly decreased the ovarian weight and number of healthy follicles. No such change was observed after 1 and 5 days of treatment except a decrease in uterus weight. Weight of liver, kidney and adrenal remained unaltered in the treated rats[47].

A similar effect of methyl parathion on ovaries along with alterations in number and duration of estrous cycle and decrease in uterus weight has also been reported although effect varied with doses[48].

Impairment of the gametogenic and cholinergic functions in a bird species, white throated munia, by methyl parathion has been reported by Maitra and Sarkar[49].

Studies on the toxicity of quinalphos, a widely used pesticide in agriculture have suggested that it is either due to the parent compound or its metabolite(s) produced prior to P-450 oxidation and induction of P-450 system[50]. Pre-treatment of rats with cobalt chloride enhanced the mortality to 100% at LD_{50} dose of the pesticide however, 3-methylcholanthrene or phenobarbitone could prevent mortality. The study suggested role of cytochrome P-450 and antioxidant enzymes in the toxicity of quinalphos.

Metasystox, an organophosphate was found to be toxic and produced hypercholinergic manifestation and haematological changes, in male calves. They died within three weeks[51].

Acute toxic effects of chlorpyriphos, a broad spectrum organophosphate were studied on goats 24 hours after the exposure. The pesticide had moderate to marked potential to exert pathophysiological effects on the body[52]. Significant reduction in litter size, pup body weight, crown rump length and trans umbilical measurements were reported in rats exposed to monocrotophos during gestation. Skeletal and visceral abnormalities were also observed at higher dose[52].

An ameliorative effect of vitamin C on the genotoxic and mutagenic effects of endosulfan, phosphamidon and mancozeb was reported[53,54]. The authors observed dose dependent amelioration by vitamin in the phosphamidon treated mice however, in the mancozeb and endosulfan treated animals, even double dose of vitamin C was ineffective.

Malathion was found to be immunosuppressive and stimulate cholinergic system at higher doses in white leghorn chicken. It was suggested that humoral immuno-suppression may be due to direct action of acetylcholine on the immune system or secondary to toxic chemical stress associated with cholinergic poisoning[55]. Anticonvulsant effects of diphenylhydantoin (DPH) were enhanced in mice following malathion treatment. The effect was more marked following repeated exposure to the pesticide for 7 days and was attributed due to inhibitory effect of malathion on drug metabolizing enzymes *viz.* aniline hydroxylase and aminopyrine N demethylase. Levels of DPH increased both in brain and liver following malathion treatment[56].

Acute effect of dichlorvos at different doses and time was studied on plasma and RBC's cholinesterase activity in mice. Cholinesterase activity in plasma was markedly inhibited and changes in the cholinesterase activity completely recovered after 48 hrs. both in plasma and RBCs[57].

Increased blood urea, GOT and GPT and decreased cholinesterase activity in rats following exposure to monocrotophos was reported[58]. These investigators suggested that the pesticide has cardiotoxic, hepatotoxic and nephrotoxic potential.

1.5. Other Pesticides

Treatment of Vepacide, an azadirachtin based neem pesticide at sub acute doses *i.e.* low (80 mg/kg/day), moderate (160 mg/g/day) and high (320 mg/kg/day) for 90 days in rats caused time, dose and tissue specific inhibition in glutathione-s-transferase, reduced glutathione and UDP-glucuronyltransferase activity in liver, lung, kidney and brain[59]. Sub-acute doses also showed a decrease in brain cytochrome P-450 level at 320 mg/kg body weight at 90th day of exposure. Decrease in cytochrome P-450 reductase activity in liver and brain was also observed after 45 and 90 days at medium and high doses[60].

Decreased body weight gain, feed intake, haemoglobin, haematocrit and RBC count following exposure to Vepacide at sub acute doses for 90 days was reported[61], suggesting toxic stress in the treated rats especially on blood and blood forming elements.

Neem based pesticide formulation Tric Vet Care, was found to affect the brain and liver biochemical parameter in rats suggesting membrane alterations and a potential of the formulation to influence the oxidant defense mechanism[62].

Acute oral LD_{50} of aluminium phosphide, a pesticide often used for suicidal purposes, in male Wistar rats was found to be 14.8, 14.5 and 13.9 mg/kg body weight by the Litchfield, Probit and Weil methods respectively[63]. The animals exhibited toxic symptoms like paraesthesia, paralysis, convulsions and coma prior to death. An immediate fall in blood pressure following intragastric administration of aluminium phosphide was observed in rats suggesting its cardiotoxic potential. The changes recovered partially and stabilized after 10 minutes at low doses (10 and 20 mg/kg) however, no recovery was seen at 40 mg/kg dose[64]. Impairment in the cellular metabolism of the myocardium as well as necrosis of the cardiac tissue due to release of reactive oxygen intermediates was suggested as the possible mechanism of its cardiotoxicity.

Low toxic potential of Stomp 30 EC (pendimethalin), a dinitroaniline weedicide, has been reported in rats[65]. In another study, moderate effects on haemopoeitic system in rats but no pathophysiological effects were observed following exposure to pendimethalin[66]. Toxic effects of hexachloro-phene, an antimicrobial agent used in medicated soaps, antiseptic solutions and various drugs were studied in rats. Activity of cytochrome oxidase was significantly decreased while that of Mg^{2+}ATPase, Ca^{2+}ATPase, & Na^+, K^+ATPase increased in liver, kidney, brain and muscle. The study indicates an inefficient electron transport system and altered calcium homeostasis in exposed rats[67].

2. Metals

Exposure to metals predominantly occurs during work in industries. However, low level exposure could occur through food chain due to widespread contamination of the environment and use of metal containing material. A number of studies on the toxic effects of metals have been undertaken during the period.

2.1. Chromium

Administration of Cr(VI) to female mice through drinking water was found to affect ovarian physiology and rate of ovulation[68] and a dose dependent reduction in the number of follicles at different stages of their maturation[69]. The metal was also found to be feto- and embryotoxic as exhibited by significant reduction in the number of implantations in the exposed animals[70]. Fetotoxicity due to exposure of pregnant mice to Cr (VI) has also been reported in mice[71].

2.2. Cadmium

Rats exposed to Cd (0.90 mg/kg, *i.p.*) for 30 days showed biomembrane changes in different brain regions. The effects of Cd were region specific and most pronounced in olfactory bulb[72].

Cadmium-induced blood brain barrier dysfunctions have been reported due to depletion of microvessel antioxidant substances and increased lipid peroxidation[73].

2.3. Vanadium

Vanadate and lead were reported to inhibit sarcolemmal and intestinal brush border membrane enzymes in a non competitive manner[74]. Interaction of vanadate with skeletal muscle sarcolemma and basement membrane indicate the presence of more than one binding site[75].

2.4. Lead

Lead, ethanol or their combination was found to decrease the levels of Cd and Mg in various tissues of rats. Exposure to Pb under condition of iron deficiency was found to reduce levels of putrescine, spermidine and spermine. These changes in physiological concentrations of polyamines by iron deficiency and lead treatment during the growth spurt period may interfere with the migration and maturation of both neuronal and glial cells resulting in perturbed neurobehavioural sequelae[76].

2.5. Manganese

Kumar *et al*[77] reported alteration in membrane properties in rat brain following exposure to manganese as evident by alterations in total phospholipid and membrane fluidity. The changes were more pronounced in young rats.

2.6. Tin

Dose and time dependent toxic effects of dibutyltin dilaurate were studied on rats[78]. Degenerative changes in periportal hepatocytes, accumulation of inflammatory cells around hyperplastic bile ducts, proliferation and occlusion of bile ducts suggested that this compound may cause cholangiohepatitis[78].

Single and multiple transplantation of fetal hippocampal cells restored trimethyltin induced hippocampal degeneration in rats[79]. The study also suggested that multiple site transplants provide better restoration on long term basis as observed by its effect on learning ability and muscaranic receptor binding.

2.7. Aluminium

Aluminium has been reported to be a non competitive inhibitor of rat brain mitochondrial monoamine oxidase in an *in vitro* study. The metal was found to form a strong chelate with the substrate, kynuramine and alter the maximum velocity (V_{max}) of the enzyme with no effect on the affinity (Km)[80].

Male rats treated with alum for 20 weeks exhibited a significant decrease in serum testosterone levels and sperm counts. Structural abnormalities, observed by histopathological studies were also reported in these rats suggesting aluminium mediated testicular damage[81].

2.8. Copper

Excess accumulation of copper in nucleus and decrease in catalase activity has been shown to be associated with DNA fragmentation in hepatocyte of Indian childhood cirrhosis disease[82].

2.9. Beryllium

Degenerative and necrotic changes coupled with a decrease in the activity of alkaline phosphatase and adenosine triphosphatase and glycogen content in liver, kidney, lungs and spleen were reported in beryllium treated rats. The changes were reversed in rats treated with LIV-52, a herbal drug[83].

3. Chelating agents

Heavy metals like lead, cadmium and mercury are known to cause varying degree of toxicity resulting in altered behavioural, biochemical and morphological parameters. In the management of metal poisoning generally chelating agents are the drugs of choice but often their use leads to the removal of the essential elements also. Therefore, attempts were made to investigate the efficacy of a combination of chelating agents and amino acids/vitamins in reducing the body burden of the metals in experimental animals and also to see if these agents alone or in combination could restore the altered biochemical parameters.

Combined treatment with calcium disodium-EDTA and meso 2,3-dimercaptosuccinic acid (DMSA) in rats was found to reduce the lead levels in liver and blood. However, renal lead levels were found to be increased with combined treatment and further investigations on such treatment were stressed upon[84]. Combination of calcium disodium versanate and DMSA was found to be effective in removing lead from other body organs as well[85]. Effect of combined treatment with thiamine and DMSA or 2,3-dimercaptopropane 1-sulphonate on the mobilization and distribution of lead in rats was also reported[86]. Chelation of lead in rats was also studied following supplementation of zinc and methionine[87].

$CaNa_2EDTA$ or DMSA was reported to be more effective than 2,3-dimercapto-1-propane-sulfonic acid (DMPS). The combination of $CaNa_2EDTA$ and DMSA was more promising than of $CaNa_2$-EDTA and DMPS or the individual chelators in restoring lead induced changes in blood and urine delta-aminolevulinic acid and blood zinc protoporphyrin levels[88]. Subsequently, it was reported that combination of $CaNa_2EDTA$ and DMSA at doses 0.2mmol/kg and 0.4mmol/kg respectively was most effective in removal of lead from brain and other body tissues[89].

Amphipathic dithiocarbamates, which are effective chelators of Cd were found to be quite effective in reducing hepatic and renal but not brain lead levels. The treatment with N-benzyl-D-glucamine dithiocarbamate and its methyl analog caused depletion of endogenous essential elements such as copper, zinc and calcium[90]. Amphipathic dithiocarbamate was also reported to mobilize metallothionein bound cadmium which was excreted in feces and prevented nephrotoxicity. N-(4-methoxybenzoyl)-D-glucamine dithiocarbamate was found to be a better inducer of hepatic metallothionein than N-benzyl-D-glucamine dithiocarbamate in removing the toxic metal from the initial circulation[91].

Administration of methionine to rats during chelation therapy was suggested to be beneficial in cadmium intoxication[92]. Comparative evaluation of various chelating agents in the amelioration of acute cadmium toxicity revealed reasonable effectivity of dithiocarbamate in mobilizing cadmium from liver and kidney of rats[93]. Combination of Vitamin E with heavy metals chelators (MFA and $CaNa_3$ DTPA) was found to be more effective than either the vitamin or the chelator in reducing body burden of Cd in rats, but was of limited advantage in reversing cadmium induced biochemical alterations[94]. Triethylene tetramine hexaacetic acid encapsulated in sphingomyelin: cholesterol liposomes exhibited high efficacy in mobilizing cadmium from the body of pre-exposed mice[95]. Pre-treatment of rats with chelators (2,3-dimercaptosuccinic acid and alpha-mercapto-beta-(2-furyl) acrylic acid) and/or zinc exhibited a variable response to cadmium administration, on blood and tissue levels of zinc, copper, calcium and magnesium[96].

A decrease in the concentration of trace elements (sodium, potassium, magnesium, manganese, copper, zinc, chromium and nickel) in brain, liver, spinal cord and kidney, except an increase in copper in liver and sodium in the kidney was reported in methylmercury treated mice. Treatment with thiols and vitamins restored the levels of these elements towards normal although fluctuations were noticed in the concentration and attributed to their association with various macromolecules[97].

$CaNa_2EDTA$ either alone or in combination with zinc resulted in a significant recovery in immunological parameters like primary antibody forming cells to T-dependent antigen and the delayed type hypersenstivity response to bovine albumin in lead treated rats[98]. Dose dependent effects of zinc supplementation as a chelator in lead treated rats was reported[99].

Effect of two chelating agents, DMSA and DMPS on the mobilization and distribution of beryllium from the hepatic and haemopoietic system was reported in rats[100]. DMPS was found to be more effective as compared to DMSA in restoring beryllium induced toxicity. Effect of thiol chelators on the beryllium induced biochemical alterations was also studied[101].

In a study on Indian childhood cirrhosis it was reported that the disease is not due to an inborn error of copper metabolism but treatment with penicillamine at an early stage in the affected cases may be useful[102].

During cadmium intoxication, zinc supplementation alongwith administration of chelating agents was found to be effective[103].

Treatment with GSH, vitamin E and selenium in combination inhibited the uptake and distribution of cadmium in rat liver and kidney[104]. GSH was found to be more effective than vitamin E and selenium and suggested to be a possible cadmium chelator. Vitamin E however was found to exacerbate Ni toxicity in mice[105].

To investigate the efficacy of structurally different six chelating agents used, alpha-mercapto-beta-(2-furyl) acrylic acid (MFA), DMSA, and N-benzyl-D-glucamine dithiocarbamate were reported to be more effective in comparison to their homologs alpha - mercapto-beta(2-thienyl) acrylic acid (MTA), DMPS, diethyldithiocarbamate respectively in increasing the urinary and fecal excretion of nickel and decreasing tissue burden of nickel[106].

Treatment with zinc, ascorbic acid or their combination during lead exposure has been reported to be more effective than post lead exposure since it could restore lead induced loss of zinc and ascorbic acid. The study suggested that these adjuvants influenced more through decrease in the absorption of lead than its mobilization from *in vivo* accumulation[107]. Lysine and zinc together were found to reduce the tissue accumulation of lead and prevent its toxic effects in rats[108].

Effect of arsenic on certain biochemical indices and therapeutic efficacy of certain chelators has been reported in blood, liver, kidney and brain in rats. Interaction of arsenic-alcohol exposure has also been studied[109-111].

Acute toxic effects of arsenic (III) were reported to be prevented in mice by DMSA, DMPS and SAM with DMSA being most effective[112]. A significant increase in brain arsenic concentration and altered neurotransmitter levels was reported in rats treated with sodium arsenite (100 ppm in drinking water) for 16 weeks. The chelation therapy with DMSA, DMPS, dimethyl-DMSA or diisopropyl-DMSA, twice daily (50 mg/kg, *i.p.*) for 5 days reduced arsenic burden however no effect on the biochemical parameters was observed in these studies[113]. It was inferred that intraperitoneal administration of arsenic antidotes may not be an effective approach due to their lipophilic character.

Multiple exposure to gallium arsenide in rats and mice caused biochemical alterations in brain and liver while single exposure caused alterations in haematological and physiological variables[114-117]. Treatment with DMSA and dimercaptopropane 1-sulfonate was reported to recover changes caused by gallium arsenide[118].

4. Solvents

An impairment of motor and visual functions was reported among painters exposed to solvents[119]. Studies on the mechanism of benzene toxicity have shown that iron hydroquinone complex may be the toxic intermediate of benzene metabolism in bone marrow[120] and 1,2,4-benzene triol, a trihydroxy metabolite, was capable of releasing iron from horse spleen ferritin under *in vitro* condition[121].

Cytotoxic effects of perchloroethylene and preventive role of 2-deoxy-glucose and vitamin E have been reported in an experimental study on mice[122].

Industrial solvents xylene, toluene and methyl alcohol either alone or in combination were found to enhance the metabolism and excretion of xylene and toluene in the presence of methanol in the liver of co-treated rats[123].

Exposure to toluene and xylene increased blood urea concentration and decreased urinary creatinine after intraperitoneal treatment of rats at alternate days upto 30 days, suggesting their nephrotoxic potential[124].

Toxic effects of gasoline: methanol blend were studied in rats, 28 days after subcutaneous treatment at 1/40th, 1/20th and 1/10th doses. A decrease in serum proteins, particularly albumins and free sulphydryl content of kidney, an increase in lipid peroxidation in liver, increase in blood delta-amino levulinic acid dehydratase activity (δ-ALAD) and a dose dependent enhancement of alkaline phosphotase activity in liver, serum and lungs was reported compared to control group and also with those treated with methanol (10% v/v) and gasoline alone[125].

To investigate the fluoride-ethanol interaction, combined toxic effects of fluoride and ethanol were studied in rats exposed through drinking water. A decrease in serum glutamic pyruvate transaminase and plasma cholinesterase was reported in rats exposed to fluoride alone and those exposed to fluoride and ethanol in combination respectively. The study suggested that toxic effects of fluoride and ethanol are independent and are not masked or decreased by the other[126].

5. Monomers and Plasticizers

Studies to understand the toxic potential of monomers and protective effect of certain agents on their toxicity have also been carried out. Acrylamide (30 mg/kg) was reported to affect the cerebellar functions of rats as evident by an impairment in the rotarod performance, locomotor activity, glutathione-S-transferase activity and cerebellar benzodiazepine receptors, 3 weeks after treatment[127]. Transplantation of dissociated fetal cerebellar cells to cerebellum resulted in a significant recovery in behavioural and neurochemical parameters, evaluated 9 weeks after transplantation. Acrylamide induced immunosuppression and its modulation by 6-MFA, an interferon inducer in rats was reported[128].

Effect of protein malnutrition on the neurobehavioural toxicity of styrene was studied in young rats[129]. A marked increase in striatal dopamine and frontocortical serotonin receptors was observed in the protein malnourished rats exposed to styrene and the changes were correlated well with behavioural alterations[129].

Studies on the toxic effects of phthalic acid esters on liver have been reviewed[130]. Differences in the sensitivity of the P-450 monooxygenases towards di(2-ethyl hexyl)phthalate (DEHP) and its metabolites amongst 3,6 and 10 weeks old rats suggesting age related effects of the plasticizer in rats were reported[131]. Enhanced vulnerability of the testes to DEHP was observed in developing rats due to impaired metabolism of the plasticizer by liver[132].

6. Food colours and dyes

Health and nutritional status of food colours was reviewed[133] and it was reported that both synthetic and natural food colours may induce allergic reactions in sensitive and atopic individuals.

Toxic effects of metanil yellow, orange II and their blend, when given parenterally to rats induced the level of cytochrome P-450 IA 1/2 protein, cytosolic GST and quinone reductase[134]. The degradation pattern of non-permitted food dyes *viz.* metanil yellow, orange II, malachite green and rhodamine B has been reported in rat caecal microflora[135,136]. Malachite green and rhodamine B were found to be biotransformed to different metabolites by rat caecal microflora which may possibly be responsible for their carcinogenic effects[135]. Metanil yellow has been shown to act as an inducer of a specific form of microsomal P-450 and cytosolic glutathione-s-transferase and quinone reductase. The changes in cytosolic enzymes in liver were more pronounced in comparison to intestine[137]. Enteric bacteria has been shown to possess both reductive and hydrolytic activity on metanil yellow and indigo carmine, and the study suggested that gut microflora may cause health problems to consumers[138].

Genotoxic effects of malachite green has been shown in Syrian hamster embryo (SHE) cells and antioxidant enzymes were found to prevent the toxic effects of the dye[139]. The dye was also found to be cytotoxic[140] and enhance the N-nitrosodiethylamine induced hepatic preneoplasia in rats in a dose dependent manner[141].

Benzanthrone dye was found to exert adverse effects on liver, kidney and testes in guinea pigs and led to normocytic anemia, reduced RBC counts. Supplementation of ascorbic acid prior to bezanthrone administration, attenuated the toxic effects of the dye[142].

Interspecies differences in the microbial reductase activity has been reported due to variation in the composition of microflora in the GI tract which may lead to altered actions in the toxic response of dyes[143]. Studies were also carried out to investigate the rate of formation of toxic aromatic amines due to anaerobic reduction of red azo dyes *viz.* amaranth, carmoisine, fast red E and ponceau 4R using caecal content and hepatic microsomal fraction of rats[144]. The study showed that caecal flora had 17 times higher degradative capability of ponceau 4 R and fast red E than the hepatic microsomal fraction indicating formation of more aromatic amines[144].

In another study on two red dyes, rhodamine was found to be genotoxic while amaranth had no effect on the somatic and germ cell lines of Drosophila[145].

Toxicity of triphenylmethane dyes in different organisms have also been reported[146].

7. Drugs, pharmacological and physiological agents

Cytotoxicity and enzyme release is being pursued in greater detail such as functional and structural organisation of cytoskeleton, plasma membrane and organelles[147-149] using various pharmacological agents, *e.g.*, dibucaine, indomethacin *etc.*, and drugs such as diazepam, phenobarbitone and others.

Activity of hepatic glutathione-S-tranferase (GST) decreased following 4 days treatment with phenobarbitone (50 mg/kg). However, no significant effect on the activity of the enzyme was observed on kidney in rhesus monkey. The observation is interesting since the drug is a well known inducer of GSTs in rodents[150]. Treatment with phenytoin produced psychomotor abnormalities in rats[151]. The study also showed that treatment with nimodipine or combination of nimodipine and phenytoin at equipotent doses produced less psychomotor side effects than phenytoin alone[151].

Sudden break in continued use of diazepam produced withdrawal reactions and also caused neuroexcitation as evident by a decrease in brain GABA levels[152]. The study also reported that these changes reversed with clonidine and propranolol.

Pharmacokinetic and renal excretion of gentamicin in goats was studied[153]. A dose regimen of 1.8 and 1.5 mg/kg, intravenously, was reported normal dose since it maintained the plasma concentrations of gentamicin within the safe therapeutic range.

Treatment of rats with pethidine at doses 5 and 7.5 mg/kg for 30 days caused a dose dependent decrease in the testis weight and inhibited the diameter of testis and seminiferous tubules[154].

The tumor inhibitory effect of low doses of butyric acid, a physiological compound, was probably due to altered epigenetic pathways[155].

Synergistic action of commonly used antibiotics and UV radiation exhibited strong superoxide radical generation potential in the following order: benzylpenicillin> amphotericin > ampicillin > nystatin > gentamycin[156]. Staphylococcus protein A being a potent immunomodulator was unable to reduce the lung lesions produced in mice by bleomycin, an antitumor antibiotic drug[157]. UVB exposure to eye was found to lead to considerable changes in maintaining the critical balance of intracellular calcium and the antioxidant defence mechanism[158]. The protective effect of a zinc chelate of histidine against cisplatin induced nephrotoxicity and gastrointestinal toxicity have been shown by decrease in blood, urea, nitrogen (BUN), creatinine and lower incidence of diarrhoea[159]. Cisplatin treatment of rats resulted in a significant increase in the activity of Ca^{2+}-independent nitric oxide synthase in kidneys and liver[160].

Differences in DNA damage by hydrogen peroxide and organic hydroperoxide/peroxide have been ascribed to the differential reactivity of DNA with hydroxyl and alkoxy/aryloxy free radicals produced respectively from these inorganic and organic peroxides[161].

Administration of inactivated *E.coli* to rats increased the permeability of blood brain barrier significantly. The study suggests that during infection NOS inductions cause the release of large quantities of NO, resulting in increased blood brain barrier permeability[162]. A study of anaesthetic ether exposure to rat showed that a single exposure caused leakage of proteins from brain mitochondria, a high rate of lipid peroxidation, and a significant increase in cytosolic Cu-Zn superoxide dismutase, thus indicating antioxidant defences to be early targets of toxic manifestations[163].

Oxidative stress produced by monosodium glutamate in neonatal rat brain regions could persist for prolonged periods[164] also, this could be one of the mechanisms of excitatory amino acid (glutamate) neurotoxicity resulting in chronic neurodegeneration.

Propolis, a natural waxy mixture produced by honey bee could significantly protect the liver from paracetamol toxicity[165]. In another study on rats, hepatotoxic effects of alcohol and carbontetrachloride were protected by propolis. Multifocal necrotic changes produced by alcohol and carbontetrachloride were also minimised following treatment with propolis[166].

Protective effect of diltiazem, a calcium channel blocker against cadmium induced hepato-renal dysfunctions in rats has been reported[167].

Pretreatment with alpha-tocopherol for 90 days has been reported to protect experimental myocardial infarction produced by isoproterenol in rats by preventing lipid peroxidation and overloading of myocardium with lipids[168].

Rats exposed to tannic acid for 7 days were reported to have decreased DNA, RNA, proteins and phosphate and it was suggested that tannic acid may cause genetic damage by affecting the rate of replication and translation[169].

An increase in lipid peroxidation and activity of glutathione-s-transferase, glutathione reductase and glutathione peroxidase in liver was reported following exposure to benzo(a)pyrene in rats. It was suggested that benzo(a)pyrene produces significant changes in GSH redox system which helps in the prevention of benzo(a)pyrene induced oxidative cell damage[170].

Guanidine has been reported to influence the kinetic behaviour of rat hepatic and renal LDH. The study showed increased affinity of the enzyme to GuHCl and not to the substrate and indicate that renal enzyme is relatively more sensitive to the IC_{50} of GuHCl than the hepatic enzyme[171].

Diminazene, used for the treatment of trypanosomiasis and babesiosis was found to damage liver of rabbits and the changes were dose dependent[172].

In vitro effect of benzo(a)pyrene, 3,3' diamino benzidine and benzanthracene, the three carcinogens, was studied on the binding of ribosomes to degenerated microsomes. Benzo(a)pyrene damaged binding sites both on polysomes and chemically degranulated microsomes and this effect may be differentiated from 3,3'-diaminobenzi-dine which does not damage the binding sites on stripped microsomes but substantially damages them on polysomes. Interestingly, benzanthracene, a week carcinogen, does not damage the binding sites either on reticular membranes or polysomes[173].

Proconvulsive action of buspirone on the chemical induced seizures with picrotoxin and role of central α_2-adrenoceptors in mice has been reported[174].

Aqueous extract of *Andrographis paniculata* was reported to be hepatoprotective and exhibited a delay in tumorigenic condition in mice[175].

In a sub-acute toxicity study on rats, Prostina, a polyherbal drug used in the treatment of benign prostatic hypertrophy, did not exhibit toxic effects upto the dose of 450 mg/kg which is 15 times higher than the recommended dose[176].

8. Other Agents

Argemone oil has often been reported to be mixed with musturd oil and outbreaks of poisoning commonly referred as "Epidemic dropsy" have been reported from time to time. Clinicoepidemiological, toxicological and safety evaluation studies on argemone oil have been reviewed[177]. Hepatic microsomal and mitochondrial lipid peroxidation was found to be enhanced in laboratory animals following exposure to argemone oil and involvement of singlet oxygen and hydroxyl radical suggested in its toxicity. Bioantioxidants was found to protect the toxicity of argemone oil.

Unilateral lesioning with 6-hydroxydopamine (6-OHDA) into caudate nucleus impaired neuro-behavioural, neurochemical and electrophysiological functions in rats which were restored eight weeks after embryonic cell transplantation[178].

Signs of hepatotoxicity as observed by focal degenerative changes in the hepatocytes and fatty changes were reported in guineapigs exposed dermally to 4%

paraphenylene diamine, an active component of the hair dye for six months. Formation of fibrinous thrombi in the blood vessels leading to oxygen deficiency in the tissues and qualitative changes in DNA content of the nuclei of the hepatocytes and stored glycogen molecules in the cytoplasm were also reported in the treated animals[179].

Effect of 1-chlororoacetophenone and dibenz (b,f)-1,4 oxazepine vapours, the two agents used extensively in tear gas to control riots was studied on rats. It was found that 1-chlororoacetophenone induced hypotension through central muscaranic receptors while dibenz (b,f)-1,4 oxazepine had less effects than the former and these were through peripheral muscaranic receptors[180].

Studies on the genotoxic potential of DMSO extract of "Pan Masala" with and without tobacco were carried out *in vitro* in the presence and absence of metabolic activation. The extracts increased the frequency of cytogenetic markers such as chromosome aberration, sister-chromatid exchange and micronucleated cells in cultures without metabolic activation. In contrast, cultures with activation exhibited a weak response and the study suggested that Pan masala contains DMSO soluble direct acting mutagen[181].

'Alta', a scarlet red solution used by some Indian women as a cosmetic to color their feet, was found to cause depigmentation of the skin. It was found to contain brilliant crocein and rhodamine B dyes[182].

Rabbits fed with edible linguda (*Diplozium esculentum*), a low level fern, exhibited systemic toxicity and died following 11 days post feeding. Decrease in body weight and organ weights, alternations in haematological parameters with degenerative changes and haemorrhages in brain and visceral organs and disrupted myocardiol fibres in heart were reported in treated rabbits[183].

Studies on the effect of common vegetables and species on hepatic microsomal and cytosolic drug metabolizing enzymes and carcinogen-DNA binding showed that dietary constituents without fractionation and purification may induce the drug metabolizing enzymes and also lower the carcinogen activity in rats fed gogu, cabbage, onion and mustard. It also showed that toxic effects of pollutants could be ameliorated due to intake of these vegetables and spices[184].

In vitro mast cell system was compared with *in vivo* systemic toxicity in mice to evaluate the cytotoxicity of biomaterial extracts and was found suitable[185].

Aflatoxicosis has been reported in broiler rabbits of White Giant, Gray Giant and Soviet Chinchille breeds maintained in ICAR animal colony[186]. The toxic effects were correlated with the presence of significant levels of aflatoxins (0.25 ppm) in the feed samples consumed by those rabbits[186]. In another study, treatment of rats with aflatoxin impaired the drug metabolizing enzymes and the effect was more pronounced on phase I enzymes compared to phase II. The study suggests that this could render the hosts more susceptible to the toxic response of chemicals[187].

9. Conclusion

Even a cursory glance of the literature in toxicology would reveal that the subject is preparing itself for another dimensional change, the major thrust being the multidisciplinary emerging frontier of toxicology. From essentially a data generation process on the gross effects, mainly for regulatory purposes, toxicology research is becoming more oriented towards understanding the mechanisms of drugs, chemicals and natural toxins toxicity. The accent, now is on getting specific clues on the mechanisms of initiation and propagation of the toxic process and biological defenses against this so that diagnostic and curative measures could emerge. Although the discipline of toxicology has been introduced at the postgraduate level in few universities, adequate facilities with modern instruments and techniques will provide an impetus to the new generation and help in developing trained manpower.

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REFERENCES

1. Anand M, Meera P, Kumar R, Gupta GSD, Tripathi O, Srimal RC. Possible role of calcium in the cardiovascular effects of prolonged administration of gamma-HCH (lindane) in rats. *J Appl Toxicol* 1995;**15**:245-8.

2. Siddiqui MKJ, Nigam U, Kaul PP, Seth TD. Bioaccumulation of HCH isomers in different tissues of young and old rats: a comparison. *Bull Environ Contam Toxicol* 1996;**56**:896-902.

3. Srivastava AK, Gupta BN, Bihari V, Mathur N, Pangtey BS, Bharti RS. Chronic effects of hexachlorocyclohexane exposure: clinical hematologic and electrocardiographic studies. *Vet Human Toxicol* 1995;**37**:302-5.

4. Agrawal D, Subramaniam A , Afaq F. Influence of hexachlorocyclohexane on phosphoinositides in rat erythrocyte membranes and brain. *Toxicol* 1995;**95**:135-40.

5. Samanta L, Chainy GB. Hexachlorocyclohexane-induced changes in lipid peroxidation, superoxide dismutase and catalase activities and glutathione content in chick liver. *Indian J Exp Biol* 1995;**33**:131-3.

6. Samanta L, Chainy GB. Age related differences of hexachlorocyclohexane effect on hepatic oxidative stress parameters of chicks. *Indian J Exp Biol* 1997;**35**:457-61.

7. Srivastava SC, Kumar R, Prasad AK, Srivastava SP. Effect of hexachlorocyclohexane (HCH) on testicular plasma membrane of rat. *Toxicol Lett* 1995;**75**:153-7.

8. Samanta L, Chainy GB. Comparison of hexachlorocyclohexane-induced oxidative stress in testis of immature and adult rats. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol* 1997;**118**:319-27.

9. Prasad AK, Pant N, Srivastava SC, Kumar R , Srivastava SP. Effect of dermal application of hexachlorocyclohexane (HCH) on male reproductive system of rat. *Hum Exp Toxicol* 1995;**14**:484-8.

10. Shukla S, Upreti RK, Raizada RB, Srivastava MK, Kidwai AM. Evaluation of technical HCH residues in differentiating rat intestinal epithelial cells. *Bull Environ Contam Toxicol* 1996;**56**:617-21.

11. Shukla S, Upreti RK , Kidwai AM. Infusion of technical HCH and fastng feeding on rat intestinal epithelial cell lining. *Indian J Toxicol* 1996;**3**:15-24.

12. Kumar R, Pant N, Srivastava SP. Hexachlorocyclohexane and its isomers: regional brain levels in the rat after dermal exposure. *Arch Environ Contam Toxicol* 1996;**35**:674-9.

13. Koner BC, Banerjee BD, Ray A. Organochlorine pesticide-induced oxidative stress and immune suppression in rats. *Indian J Exp Biol* 1998;**36**:395-8.

14. Banerjee BD, Saha S, Mohapatra TK, Ray M. Influence of dietary protein on DDT-induced immune responsiveness in rats. *Indian J Exp Biol* 1995;**33**:739-44.

15. Banerjee BD, Koner BC, Ray A. Influence of stress on DDT-induced humoral Immune responsiveness in mice. *Environ Res* 1997;**74**:43-7.

16. Banerjee BD, Ray A, Pasha ST. A comparative evaluation of immunotoxicity of DDT and its metabolities in rats. *Indian J Exp Biol* 1996;**34**:517-22.

17. Diwivedi PCD., Singh SP, Sharma LD. Acute toxicity of endosulfan in calves with reference to haematobiochemical changes. *Indian J Toxicol* 1995;**2**:13-9.

18. Sinha N, Narayan R , Saxena DK. Effect of endosulfan on the testis of growing rats. *Toxicol Lett* 1996;**89**:147-54.

19. Subramoniam A, Khanna VK, Mohindra J, Seth PK. Reduced phosphoinositide levels in endosulfan treated rat brain. *Indian J Pharmacol* 1994;**26**:49-51.

20. Banerjee BD, Koner BC, Ray A. Immunotoxicity of pesticides: perspectives and trends. *Indian J Exp Biol* 1996;**34**: 723-33.

21. Banerjee BD, Koner BC, Ray A, Pasha ST. Influence of subchronic exposue to lindane on humoral immunity in mice. *Indian J Exp Biol* 1996;**34**:1109-13.

22. Agrawal RC, Kumar S , Mehrotra NK. Micronucleus induction by diuron in mouse bone marrow. *Toxicol Lett* 1996;**89**: 1-4.

23. Agrawal RC, Shukla Y , Mehrotra NK. Assessment of mutagenic potential of thiram. *Food Chem Toxicol* 1997;**35**: 523-5.

24. Agrawal RC, Mehrotra NK. Assessment of mutagenic potential of propoxur and its modulation by indole-3-carbinol. *Food Chem Toxicol* 1996;**35**:1081-4.

25. Mishra VK, Srivastava MK, Raizada RB. Testicular toxicity in rat to repeated oral administration of tetramethylthiuram disulfide (Thiram). *Indian J Exp Biol* 1998;**36**:390-4.

26. Pant N, Shankar R, Srivastava SP. *In utero* and lactational exposure of carbofuran to rats: effect on testes and sperm. *Hum Exp Toxicol* 1997;**16**:267-72.

27. Kackar R, Srivastava MK, Raizada RB. Induction of gonadial toxicity to male rats after chronic exposure to mancozeb. *Indus Health* 1997;**35**:104-11.

28. Kackar R, Srivastava MK, Raizada RB. Studies on rat thyroid after oral adminstration of mancozeb: morphological and biochemical evaluation. *J Appl Toxicol* 1997;**17**:369-75.

29. Pant N, Shankar R , Srivastava SP. Spermatotoxic effects of carbaryl in rats. *Hum Exp Toxicol* 1996;**15**:736-8.

30. Khurana R, Mahipal SK, Chauhan RS. Effect of Carbofurna on cell mediated immunity in sheep. *Indian J Toxicol* 1998;**5**: 1-6.

31. Khurana SK, Chauhan RS, Mahipal SK. Immunotoxic

effect of cypermethrin on humoral immune response in chickens. *Indian J Toxicol* 1998;**5**:37-40.

32. Ayub Shah MA, Gupta PK. Influence of permethrin a synthetic pyrethroid insecticide on immune responses of mice. *Indian J Toxicol* 1998;**5**:13-9.

33. Ayub Shah MA, Gupta PK. Biochemico-toxicological study on permethrin a synthetic pyrethroid insecticde in rats. *Indian J Toxicol* 1997;**4**:57-60.

34. Husain R, Husain R, Adhami VM , Seth PK. Behavioural, neurochemical and neuromorphological effects of deltamethrin in adult rats. *J Toxicol Environ Hlth* 1994;**48**: 515-26.

35. Husain R, Malaviya M, Seth PK, Husain R. Effect of deltamethrin on regional brain polyamines and behaviour in young rats. *Pharmacol Toxicol* 1994;**74**:211-5.

36. Garg SK, Shah Ayub MA, Garg KM, Farooqui MM, Sabir M. Biochemical and physiologcial alterations following short term exposure to fluvalinate-a synthetic pyrethroid. *Indian J Pharmacol* 1997;**29**:250-4.

37. Kaul PP, Rastogi A, Hans RK, Seth TD, Seth PK, Srimal RC. Fenvalerate-induced alterations in circulatory thyroid hormones and calcium stores and rat brain. *Toxicol Lett* 1996;**89**:29-33.

38. Maiti PK, Kar A. Dual role of testosterone in fenvalerate-treated mice with respect to thyroid function and lipid peroxidation. *J Appl Toxicol* 1997;**17**:127-31.

39. Vandana S, Zzaman S. Phorate-induced enzymological alterations in mouse olfactory bulb. *Brain Res Bull* 1997;**44**: 247-52.

40. Maiti PK, Kar A. Dimethoate inhibits extrathyroidal 5'-monodeiodination of thyroxine to 3,3', 5-triiodothyronine in mice: the possible involvement of the lipid peroxidative process. *Toxicol Lett* 1997;**91**:1-6.

41. Srivastava MK, Raizada RB. Developmental effect of technical dimethoate in rats: Maternal and fetal toxicity evaluation. *Indian J Exp Biol* 1996;**34**:329-33.

42. Reddy PN. Effect of dimethoate on the crosslinting of rat dermal collagen in ageing fed with normal and high protein diets. *Indian J Toxicol* 1994;**1**:11-6.

43. Venkateshwaralu P, Sharma BJR, Kala Kumar B, Reddy KS, Ravi Kumar P. Comparative evaluation of toxicity of carbaryl, cypermethrin and malathion on tests in mice. *Indian J Toxicol* 1997;**4**:33-7.

44. Akbarsha MA, Sivasamy P. Male reproductive toxicity of phosphamidon: histopathological changes in epididymis. *Indian J Exp Biol* 1998;**36**:34-8.

45. Vijayaraghavan M, Nagarajan B. Mutagenic potenital of acute exposue to organophosphours and organochlorine compounds. *Mutat Res* 1994;**321**:103-11.

46. Vasudev V, Krishnamurthy NB. *In vivo* cytogenetic analyses of the carbamate pesticides Dithane M-45 and Baygon in mice. *Mutat Res* 1994;**323**:133-5.

47. Asmathbanu I, Kaliwal BB. Temporal effect of methyl parathion on ovarian compensatory hypertrophy, follicular dynamics and estrous cycle in hemicastrated albino rats. *J Basic Clin Physiol Pharmacol* 1997;**8**:237-54.

48. Dhondup P, Kaliwal BB. Inhibition of ovarian compensatory hypertrophy by the administration of methyl parathion in hemicastrated albino rats. *Reprod Toxicol* 1997;**11**:77-84.

49. Maitra SK, Sarkar R. Influence of methyl parathion on gametogenic and acetylcholinesterase activity in the testis of whitethroated munia (*Lonchura malabarica*). *Arch Environ Contam Toxicol* 1996;**30**:384-9.

50. Dwivedi PD, Dasm, Khanna, SK. Role of cytochrome P450 in quinaalphas toxicity: effect on hepatic and brain antioxidant enzymes in rats. *Food Chem Toxicol* 1998;**38**:437-44.

51. Kumar N, Singh SP, Sharma LD. Clinicopathological studies on induced Metasystox toxicity in calves. *Indian J Toxicol* 1996;**3**:35-40.

52. Kaur H, Srivastava AK, Garg SK, Prakash D. Studies on pathophysiological aspect of acute chlorpyriphos toxicity in goats. *Indian J Toxicol* 1998;**5**:53-8.

53. Khan PK, Sinha SP. Vitamic C mediated amelioration of pesticide genotoxicity in murine spermatocytes. *Cytobios* 1994;**80**:199-204.

54. Khan PK, Sinha SP. Impact of higher doses of vitamin c in modulating pesticide genotoxicity. *Teratog Carcinog Mutagen* 1994;**14**:175-81.

55. Rishi S, Garg BD. Effect of malathion induced cholinergic stimulation on humoral immune response in WLH chicken. *Indian J Toxicol* 1997;**4**:27-31.

56. Kumar V, Uppal RP. Effect of malathion the anticonvulsant activity of diphenylhydantion in mice. *Indian J Toxicol* 1996; **3**:51-5.

57. Bhatnagar VK, Farma VKJ, Vora AB. Inhibition of cholinesterase in male mice fed dichlorvos (DDVP). *Indian J Toxicol* 1994;**1**:49-51.

58. Adilaxamamma K, Reddy KS. Effect of monocrotophos on blood chemistry and serum enzymic profile in lactating rats. *Indian J Toxicol* 1995;**2**:21-5.

59. Mahboob M, Siddiqui MKJ., Mustafa M. Subacute effects of a neem pesticide on some of the detoxifying enzymes of rats. *Indian J Toxicol* 1995;**2**:1-11.

60. Mahboob M, Siddiqui MK, Jamil K. The effect of subacute administration of a neem pesticide on rat metabolic enzymes. *J Environ Sci Health-B* 1998;**33**:425-38.

61. Rahman MF, Siddiqui MKJ , Mustafa M. Effect of repeated administration of Vepacide (*Azadirachtin indica*) on some haemotological and biochemical parameters in rats. *Indian J Toxicol* 1996;**3**:1-8.

62. Kataria M, Gupta PK, Gupta S, Vijjan VK. Influence of neem based pesticide formulation Tric Vet Care on biochemical parameters in rats. *Indian J Toxicol* 1998;**5**:31-6.

63. Batra K, Taenja OP, Khemani LD. Acute oral toxicity of aluminium phosphide in male albino rats (Wistar). *Bull Environ Contam Toxicol* 1994;**52**:662-6.

64. Lall SB, Sinha K, Mittra S, Seth SD. An experimental study on cardiotoxicity of aluminium phosphide. *Indian J Exp Biol* 1997;**35**:1060-4.

65. Ayub Shah MA, Garg SK, Garg KM, Farooqui MM, Alam A, Sabir M. Effect on toxicological evaluation of stomp 30 EC (*Pendimethalin*) with particular reference to hematological and hemobiochemical changes in rats. *Indian J Toxicol* 1994;**1**:17-24.

66. Ayub Shah MA, Garg SK, Garg KM, Farooqui MM, Sabir M. Chronic toxicity studies on pendimethalin in rats. *Indian J Toxicol* 1998;**5**:41-4.

67. Lokanatha V, Sailaja P, Rajendra W. Effect of hexachlorophene on the cytochrome oxidase and ATPase systems in the tissues of rat. *Indian J Toxicol* 1997;**4**:39-44.

68. Junaid M, Murthy RC, Saxena DK. Embryotoxicity of orally administered chromium in mice: exposure during the period of organogenesis. *Toxicol Lett* 1996;84:143-8.

69. Murthy RC, Junaid M, Saxena DK. Ovarian dysfunction in mice following chromium (VI) exposure. *Toxicol Lett* 1996; **89**:147-54.

70. Kanojia RK, Junaid M, Murthy RC. Chromium induced teratogenicity in female rat. *Toxicol Lett* 1996;**89**:207-13.

71. Junaid M, Murthy RC, Saxena DK. Chromium fetotoxicity in mice during late pregnancy. *Vet Human Toxicol* 1995;**37**: 320-3.

72. Kumar R, Agarwal AK , Seth PK. Oxidant stress mediated neurotoxicity of cadmium. *Toxicol Lett* 1996;**89**:65-9.

73. Shukla A, Shukla GS, Srimal RC. Cadmium-induced alterations in blood-brain barrier permeability and its possible correlation with decreased microvessel antioxidant potential in rat. *Hum Exp Toxicol* 1996;**15**:400-5.

74. Gupta K, Upreti RK , Kidwai AM. Toxicokinetic study of rat intestinal brush border membrane enzymes following *in vitro* exposure to lead and vanadium. *Bull Environ Contam Toxicol* 1994;**52**:919-26.

75. Upreti RK. Membrane-vanadium interaction: a toxicokinetic evaluation. *Mol Cell Biochem* 1995;**153**:167-71.

76. Adhami VM, Husain R, Husain R, Seth PK. Influence of iron deficiency and lead treatment on behaviour and cerebellar and hippocampal polyamine levels in neonatal rats. *Neurochem Res* 1996;**21**;921-28.

77. Kumar R, Srivastava S, Agarwal AK, Seth PK. Alteration in some membrane properties in rat brain following exposure to manganese. *Pharmacol Toxicol* 1996;**79**:47-8.

78. Khandelwal S, Shukla LJ, Shanker R. Dibutyltin dialurate induced hepathotoxicity biochemical and histological alterations in rats. *Indian J Toxicol* 1998;5:21-9.

79. Roy A, Agrawal AK, Husain R, Seth PK. Restoration of trimethyltin induced hippocampal degeneration of single and multiple transplantation. *Restor Neurol Neurosci* 1997; **11**:195-201.

80. Rao KS, Rao GV. Effect of aluminium (Al) on brain mitochondrial monamine oxidase-A (MAO-A) activity-an *in vitro* kinetic study. *Mol Cell Biochem* 1994;**137**:57-60.

81. Sharma M, Balani DK, Pillai KK, Husain SZ, Athar M. Aluminium mediated testicular damage: action through hypothalamic pituiatary axis. *Indian J Toxicol* 1996;**3**:9-14.

82. Prasad R, Kaur G, Nath R, Walia BN. Molecular basis of pathophysiology of Indian Childhood Cirrhosis: Role of nuclear copper accumulation in liver. *Mol Cell Biochem* 1996;**156**:25-30.

83. Mathur S, Mathur R, Prakash AO. Protective action of a herbal preparation Liv-52 against beryllium toxicity in rats. *Biomed Environ Sci* 1994;**7**:180-97.

84. Flora GJS, Seth PK, Prakash AO, Mathur R. Therapeutic efficacy of combined meso 2,3-dimercaptosuccinic acid and calcium disodium edetate treatment during acute lead intoxication in rats. *Hum Exp Toxicol* 1995;**14**:410-3.

85. Flora GJS, Seth PK, Flora SJS. Recoveries in lead induced alterations in rat brain biogenic amines levels following combined chelation therapy with meso 2,3-dimercaptosuccinic acid and calcium disodium versenate. *Biogenic Amines* 1997;**13**:79-90.

86. Flora SJS, Pant SC, Sachan AS. Mobilization and distribution of lead over the course of combined treatment with

Thiamine and meso 2,3-dimercaptosuccinic acid or 2,3 dimercaptopropane 1-sulphonate in rats. *Clin Chem Enzy Comm* 1994;**6**:207-16.

87. Tandon SK, Singh S, Flora SJS. Influence of methionine and zinc supplementation during chelation of lead in rats. *J Trace Elem Electrolytes Health Dis* 1994;**8**:75-7.

88. Tandon SK, Singh S, Jain VK. Efficacy of combined Chelation in lead intoxication. *Chem Res Toxicol* 1994;**7**:585-9.

89. Tandon SK, Singh S, Prasad S, Mathur N. Mobilization of lead by calcium versenate and dimercaptosuccinate in the rat. *Clin Exp Pharmacol Physiol* 1998;**25**:686-92.

90. Tandon SK, Singh S, Prasad S. Chelation in metal intoxication II: efficacy of amphipathic dithiocarbamates in mobilization of lead in the rat. *Hum Exp Toxicol* 1997;**16**: 557-62.

91. Tandon SK, Prasad S, Singh S, Agarwal DK. Efficacy of amphipathic dithiocarbamates in intracellular cadmium mobilization and in modulation of hepatic and renal metallothionein in cadmium pre-exposed rat. *Chem Biol Interact* 1998;**114**:161-75.

92. Tandon SK, Singh S, Prasad S. Influence of methionine administration during chelation of cadmium by CaNO3 DTPA and DMPS in the rat. *Environ Toxicol Pharmacol* 1997;**3**:159-65.

93. Srivastava RC, Gupta S, Ahmad N, Hasan SK, Farookh A, Husain MM. Comparative evaluation of chelating agents on the mobilization of cadmium : a mechanistic approach. *J Toxicol Environ Hlth* 1996;**47**:173-82.

94. Tandon SK, Singh S. Influence of vitamin E on preventive or therapeutic effect of MFA and DTPA in cadmium toxicity. *Biomed Environ Sci* 1995;**8**:59-64.

95. Behari JR, Gupta S, Srivastava S, Misra M, Srivastava RC. Implications for benzene toxicity efficacy of liposomes encapsulated triethylene tetramine hexaacetic acid (TTHA) against cadmium intoxication: role of lipid composition. *Industr Hlth* 1994;**33**:83-8.

96. Tandon SK, Singh S. Influence of zinc and chelators pretreatment on the disposition of cadmium and some essential elements in rats. *Clin Chem Enzym Comms* 1995;**6**: 315-21.

97. Bapu C, Purohit RC, Sood PP. Fluctuation of trace elements during methyl mercury toxication and chelation therapy. *Hum Exp Toxicol* 1994;**13**:815-23.

98. Kumar P, Rai GP, Flora SJS. Immunomodulation follwoing zinc supplementation during chelation of lead in male rats. *Biometals* 1994;**7**:41-4.

99. Flora SJS, Bhattacharya R, Sachan SRS. Dose dependent effects of zinc supplementation during chelation of lead in rats. *Pharmacol Toxicol* 1994;**74**: 330-33.

100. Flora SJS, Mathur S, Mathur R. Effects of meso-2, 3-dimercaptosuccinic acid or 2,3-dimercaptopropane 1-sulfonate on berylliym-induced biochemical alterations and metal concentration in male rats. *Toxicology* 1995;**95**:167-75.

101. Mathur S, Flora SJS, Mathur R, Kannan GM , Das Gupta S. Beryllium induced biochemical alterations and their response to Thiol chelators in rats. *J Appl Toxicol* 1994;**14**: 263-7.

102. Bavdekar AR, Bhave SA, Pradhan AM, Pandit AN, Tanner MS. Long term survival in Indian childhood cirrhosis treated with D-pencillamine. *Arch Dis Child* 1996;**74**:32-5.

103. Flora SJS, Gubrelay U, Kannan GM, Mathur R. Effects of zinc supplementation during chelating agents administration in cadmium intoxication. *J Appl Toxicol* 1998;**18**:357-62.

104. Rana SV, Verma S. Protective effects of GSH, vitamin E and selenium onlipid peroxidation in cadmium-fed rats. *Biol Trace Elem Res* 1996;**51**:161-8.

105. Husain MM, Ahmad N, Gupta S, Behari JR, Hasan SK, Srivastava KK , Srivastava RC. Exacerbation of nickel induced oxidative response by vitamin E. *Ind Health* 1995;**33**:143-52.

106. Tandon SK, Singh S, Jain VK, Prasad S. Chelation in metal intoxication. XXXVIII. Effect of structurally different chelating agents in treatment of nickel intoxication in rat. *Fundam Appl Toxicol* 1996;**31**:141-8.

107. Tandon SK, Jain VK. Efficacy of adjuvants in prevention or therapy of lead intoxication. *Indian J Toxicol* 1994;**1**: 25-32.

108. Tandon SK, Singh S, Prasad S, Mathur N. Influence of L-lysine and zinc administration during exposure to lead or lead and ethanol in rats. *Biol Trace Elem Res* 1997;**57**: 51-8.

109. Flora SJS, Dube SN, Kannan GM, Arora UI, Malhotra PR. Therapeutic potential of Meso 2,3-dimereapto suuinic acid and 2,3-dimercaptopropane 1-sulfonate against Chronic Arsenic poisoning in Rats. *Biometals* 1995;**8**:111-7.

110. Flora SJS, Pant SC, Malhotra PR, Kannan GM. Biochemical and histopathological changes in arsenic intoxicated rats co-exposed to ethanol. *Alcohol* 1997;**14**:563-68.

111. Flora SJS, Pant BP, Tripathi N, Kannan GM , Jaiswal DK. Distribution of Arsenic by diesters of Meso 2,3-dimercaptosuccinic acid during sub-chronic intoxication in rats.

J Occup Health 1997;**39**:119-23.

112. Tripathi N, Flora SJS. Effects of some thiol chelators and arsenic on enzymatic activities in blood, liver and kidneys in mice. *Biomed Environ Sci* 1998;**11**:38-45.

113. Tripathi N, Kannan GM, Pant BP, Jaiswal DK, Malhotra PR, Flora SJS. Arsenic induced changes in certain neurotransmitters level and their recoveries following chelation in rat whole brain. *Toxicol Lett* 1997;**92**:201-8.

114. Flora SJS. Alterations in some hepatic biochemical variables following repeated gallium arsenide administration in rats. *Internat Heaptol Commun* 1996;**5**:97-103.

115. Flora SJS, Dube SN, Pant SC. Effect of multiple gallium arsenide exposure on some biochemical indices in rat brain. *Indust Health* 1994;**32**:247-52.

116. Flora SJS, Dube SN, Pant SC, Vijayaraghavan R. Changes in certain haematological and physiological variables following single gallium arsenide exposure in rats. *Biol Trace Elem Res* 1997;**58**:197-208.

117. Flora SJS, Kumar P, Kannan GM, Rai GP. Effects of single oral administration of gallium arsenide on some selected biochemical and immunological variables in male wistar rats. *Toxicol Lett* 1998;**94**:103-13.

118. Flora SJS, Kumar P. Biochemical and immunotoxicological alterations following repeated gallium arsenide exposure and their recoveries by meso 2,3-dimercaptosuccinic acid and 2,3-dimercaptopropane 1-sulfonate administration in rats. *Europ J Pharmacol: Environl Pharmacol Toxicol Section* 1996;**2**:315-20.

119. Tripathi SR, Battacharya SK, Kashyap SK. Neurobehavioural changes in workshop painters of a public transport network. *J Hum Ergol Tokyo* 1995;**24**:153-60.

120. Singh V, Ahmad S, Rao GS. Prooxidant and antioxidant properties of iron hydroquinone and iron-1,3,4-benzenetriol complex. *Toxicol* 1994;**89**:25-33.

121. Ahmad S, Singh V, Rao GS. Release of iron from ferritin by 1,2,4-benzenetriol. *Chem Biol Interact* 1995;**96**:103-11.

122. Ebrahim AS, Babakrishnan K, Sakthisekaran D. Perchlorethylene-induced alterations in glucose metabolism and their prevention by 2-deoxy-o-glucose and vitamin E in mice. *J Appl Toxicol* 1996;**16**:339-48.

123. Rana SV, Kumar S. Antioxidative enzyme in the liver of rats after exposure to xylene, toluene and methyl alcohol spearately and in combination. *Physiol Chem Phys Med NMR* 1995;**27**:25-9.

124. Kumar S. Nephrotoxicity of toluene and xylene exposure among rats. *Indian J Toxicol* 1998;**5**:59-63.

125. Gupta GSD, Ahmad S, Goel SK, Khandelwal S. Biochemical alterations in rats following administration of gasoline: methanol blend. *Indian J Toxicol* 1997;**4**:7-14.

126. Pillai KS, Murthy PB. Toxicity of combination of fluoride and ethanol to rat. *Indian J Toxicol* 1994;**1**:69-72.

127. Husain R, Agarwal AK, Hasan M, Seth PK. Modulation of acrylamide-induced neurochemical and behavioural deficits by cerebellar transplants in rats. *Pharmacol Biochem Behav* 1994; **49**:443-48.

128. Zaidi SIA, Raisuddin S, Singh KP, Jafri A, Husain R *et al.* Acrylamide induced immunosupressin in rats and its modulation by 6-MFA, an interferon inducer. *Immunopharmacol Immunotoxicol* 1994; **16**:247-60.

129. Khanna VK, Husain R, Seth PK. Effect of protein malnutrition on the neuro behavioural toxicity of styrene in young rats. *J Appl Toxicol* 1994;**14**:351-6.

130. Parmar D, Seth PK. Phthalic acid esters and liver. In: Liver and environmental xenobiotics (Ed. SVS Rana),New Delhi, Narosa Publishing House, 1997; pp 72-91.

131. Parmar D, Srivastava SP, Seth PK. Age related effects of di(2-ethylhexyl) phthalate on hepatic cytochrome P450 monooxygenase in wistar rats. *PharmacolToxicol* 1994;**74**: 177-180.

132. Parmar D, Srivastava SP, Singh GB, Seth PK. Testicular toxicity of di(2-ethylhexyl) phthalate in developing rats. *Vet Human Toxicol* 1995;**37**:310-3.

133. Babu S, Shenolikar IS. Health and nutritional implications of food colours. *Indian J Med Res* 1995;**102**:245-9.

134. Ramchandani S, Das M, Joshi A, Khanna SK. Effect of metanil yellow, orange II and their blend on hepatic xenobiotic metabolizing enzymes in rats. *Food Chem Toxicol* 1994;**32**:559-63.

135. Singh S, Das M, Khanna SK. Biodegradation of malachite green and rhodamine B by caecal microflora of rats. *Biochem Biophys Res Commun* 1994; **200**:1 544-50.

136. Singh S, Das M, Khanna SK. Bio-metabolic disposition of metanil yellow, orange II and their blend by caecal microflora of rats. *Indian J Exp Biol* 1995;**33**:543-4.

137. Ramchandani S, Das M, Joshi A, Khanna SK. Effect of oral and parenteral administration of metanil yellow on some hepatic and intestinal biochemical parameters. *J Appl Toxicol* 1997;**17**:85-91.

138. Singh S, Das M, Khanna SK. Reductive and hydrolytic activities of enteric bacteria on food colours. *Indian J Exp*

Biol 1997;**35**:912-4.

139. Panandekar A, Maru GB, Rao KB. Dose response effect of malachite green on free radical formation, lipid peroxidation and DNA damage in syrian hamster embryo cells and their modulation by antioxidants. *Carcinogenesis* 1994;**15**:2445-8.

140. Rao KV. Inhibition of DNA synthesis in primary rat hepatocyte cultures by malachite green: a new liver tumor promoter. *Toxicol Lett* 1995;**81**:107-13.

141. Rao KV, Fernandes CL. Progressive effect of malachite green at varying concentrations on development of N-nitrosodiethylamine induced hepatic preneoplastic lesions in rats. *Tumori* 1996;**82**:280-6.

142. Das M, Garg K, Singh GB, Khanna SK. Attenuation of benzanthrone toxicity by ascorbic acid in guinea pigs. *Fundam Appl Toxicol* 1994;**22**: 447-56.

143. Singh S, Das M., Khanna SK. Azo reductase activity of microbial population from gastrointestinal tract segments of various animals species. *Indian J Exp Biol* 1998;**36**: 99-103.

144. Singh S, Das M, Khanna SK. Comparative azo reductase activity of red azo dyes through caecal and hepatic microsomal fraction in rats. *Indian J Exp Biol* 1997;**35**:1016-8.

145. Tripathy NK, Nabi MJ, Sahu GP, Kumar AA. Genotoxicity testing of two red dyes in the somatic and germ line cells of drosophilla. *Food Chem Toxicol* 1995;**33**:923-7.

146. Azmi W, Sani RK, Banerjee UC. Biodegradation of triphenylmethane dyes. *Enzyme Microb Technol* 1998; **22**:185-91.

147. Musavi S, Kakkar P. Diazepam induced early oxidative changes at the subcellular level in the rat brain. *Mol Cell Biochem* 1998;**178**:41-6.

148. Kakkar P, Mehrotra S, Viswanathan PN. BHP induced *in vitro* swelling of rat liver mitochondria. *Mol Cell Biochem* 1996;**154**:39-45.

149. Kakkar P, Mehrotra S, Viswanathan PN. Influence of antioxidants on the peroxidative swelling of mitochondria *in vitro*. *Cell Biol Toxicol* 1998;**14**:313-21.

150. Ramana KV , Kohli KK. Differential effects of phenobarbitone on the hepatic and renal glutathione-s-transferases in the rhesus monkey. *Indian J Pharmacol* 1998;**30**:34-7.

151. Balakrishnan S, Bhargava VK, Pandhi P. Effect of nimodipine on the psychomotor dysfunction induced by phenytoin in rats. *Indian J Pharmacol* 1998;**30**: 299-305.

152. Singh H, Goyal BL, Singh P. Study of neuro-excitatory features during diazepam-withdrawal reactions and their correlation with brain gamma-aminobutyric acid levels. *Indian J Pharmacol* 1998;**30**:267-70.

153. Uppal RP, Verma SP, Garg KS. Pharmacokinetics and renal excretion of gentamicin in goats after intravenous administration. *Indian J Pharmacol* 1998;**30**:47-50.

154. Patil S, Patil S, Londonkar R, Patil SB. Effect of pethidine on spermatogenesis in albino rats. *Indian J Pharmacol* 1998;**30**:249-53.

155. Gupta KP, Mehrotra NK. Differential effects of butyric acid on mouse skin tumorigenesis. *Biomed Environ Sci* 1997;**10**: 436-41.

156. Ray RS, Mehrotra S, Prakash S, Joshi PC. Ultraviolet radiation induced production of superoxide radicals by selected antibiotics. *Drug Chem Toxicol* 1996;**19**:121-30.

157. Sahu AP, Lal K. Effects of bleomycin and staphylococcus protein-A on lungs of mice. *Indian J Exp Biol* 1995;**33**:734-38.

158. Babu V, Misra RB, Joshi PC. Ultraviolet B effect on occular tissues. *Biochem Biophys Res Comm* 1995;**210**:417-23.

159. Srivastava RC, Farookh A, Ahmad N, Misra M, Hasan SK, Husain MM. Reduction of cis-platinum induced nephrotoxicity by zinc histidine complex: the possible implication of nitric oxide. *Biochem Mol Biol Intern* 1995;**36**:855-62.

160. Srivastava RC, Farookh A, Ahmad N, Misra M, Hasan SK, Husain MM. Evidence for the involvement of nitric oxide in cisplatin induced toxicity in rats. *Biometals* 1996;**9**:139-42.

161. Mahmood N, Khan SG, Athar M, Rahman Q. Differential role of hydrogen peroxide and organic peroxides in augmenting asbestos mediated DNA damage: implications for asbestos induced carcinogenesis. *Biochem Biophys Res Commun* 1994;**200**:687-94.

162. Shukla A, Dikshit M, Srimal RC. Nitric oxide modulates blood-brain barrier permeability during infections with an inactivated bacterium. *Neuro Rep* 1995;**6**:1629-32.

163. Viswanathan V, Kakkar P, Seth PK. Some early biochemical changes in rat brain after single exposure to anaesthetic ether. *J Environ Biol* 1996;**17**:1-4.

164. Bawari M, Babu GN, Ali MM, Misra UK. Effect of neonatal monosodium glutamate on lipid peroxidation in adult rat brain. *Neuro Report* 1995;**6**:650-2.

165. Sharma M, Pillai KK, Hussain SZ, Giri DK. Protective effect of propolis against paracetamol toxicity in rat liver. *Indian J Toxicol* 1998;**5**:27-34.

166. Sharma M, Pillai KK, Husain SZ, Giri DK. Protective role

of propolis against alcohol-carbon tetrachloride-induced hepatotoxicity in rats. *Indian J Pharmacol* 1997;**29**:76-81.

167. Chatterjee S, Gupta PK. Protective effect of calcium channel blocker, diltizaem against cadmium - induced hepato renal dysfunction in rats. *Indian J Toxicol* 1994;**1**:41-7.

168. Ithayarasi Paritha A, Devi Shyamala CS. Effect of α-tocopherol on isoproterenol-induced changes in lipid and lipoprotein profile in rats. *Indian J Pharmacol* 1997;**29**:399-404.

169. Sharma S, Dani HM. Derangements in transformation related biochemical expressions during initiation of hepatic carcinogenetic lesions induced in rats by tannic acid administration. *Indian J Toxicol* 1995;**2**:27-30.

170. Sharma S, Dani HM. Benzo (a) pyrene induced alterations in rat liver: lipid peroxidation, glutathione and related enzymes. *Indian J Toxicol* 1996;**3**:57-61.

171. Rao SVR, Reddy KS, Indira K. Inhibitory Kinetic responses of rat hepatic and renal lactate dehydrogenase to guanidine. *Indian J Toxicol* 1996;**3**:29-34.

172. Nanda SK, Mohapatra M, Parida S, Mishra SN. Toxic effects of diminazene on albino rats. *Indian J Toxicol* 1997;**4**: 23-6.

173. Sharma S, Dani HM. Effect of carcinogens binding of ribosomes to degranulated microsomes. *Indian J Toxicol* 1997;**4**:51-5.

174. Vohra D, Pillai KK. Proconvulsant effect of buspirone and its modulation by α_2-adrenoceptor agonist in mice. *Indian J Pharmacol* 1998;**30**:329-33.

175. Trivedi N, Rawal UM. Effect of aqueous extract of *Andrographis paniculata* on liver tumor. *Indian J Pharmacol* 1998; **30**:318-22.

176. Biswas NR, Sen S, Singh S, Gopal N, Pandey RM, Giri D. Sub-acute toxicity study of a polyherbal drug (prostina) in rats. *Indian J Pharmacol* 1998;**30**:239-44.

177. Das M, Khanna SK. Clinico-epidemiological, toxicological and safety evaluation studies on argemone oil. *Crit Rev Toxicol* 1997;**27**:273-97.

178. Agarwal AK, Husain R, Raghubir R, Kumar A, Seth PK. Neurobehavioural, neurochemical and electrophysiological studies in 6-hydroxydopamine lesioned and neural transplanted rats. *Int J Devl Neuroscience* 1995;**13**:105-11.

179. Dutta V, Devi M, Dutta K. Effect of dermal exposure to paraphenylene diamine in guineapigs. *Indian J Toxicol* 1998;**5**:21-6.

180. Kumar P, Kumar D, Vijayaraghavan R, Gupta Das S. Effect of 1-chloroacetophenone (CN) and dibenz (b,f)-1,4 oxazepine (CR) vapour inhalation on some cardiovascular and respiratory variables in anaesthetised rats. *Indian J Pharmacol* 1997;**29**:238-43.

181. Patel RK, Jaju RJ, Bakshi SR, Trivedi AH, Dave BJ *et al.* Pan masala a genotoxic menace. *Mutat Res* 1994;**320**: 245-9.

182. Bajaj AK, Pandey RK, Misra K, Chatterji AK, Tiwari A, Basu S. Contact depigmentation caused by an azo dye in alta. *Contact Dermatitis* 1998;**38**:189-93.

183. Somvanshi R, Devi V, Gounalan S, Kataria M. Preliminary clinicopathological observations on effects of linguda (*Diplazium esculentum*) feeding in laboratory rabbits. *Indian J Toxicol* 1998;**5**:7-11.

184. Rajpurohit R, Krishnaswamy K. Effect of common vegetables/spices on genobiotic metaboloizing enzymes and *in vivo* carcinogen DNA binding. *Indian J Toxicol* 1994;**1**: 33-40.

185. Rathinam K, Mohanan PV. *In vitro* mast cell system for evaluating cytotoxicity of biomaterial extracts. *Indian J Toxicol* 1996;**3**: 41-4.

186. Singh KP, Singh R. Aflatoxicosis in broiler rabbits. *Indian J Toxicol* 1998;**5**:35-9.

187. Raisuddin S, Parmar D, Zaidi SIA, Singh KP, Verma AS, Seth PK. Aflatoxin induses depletion of activities of phase I biotransformation enzymes in growing rats. *Europ J Drug Metab Pharmacok* 1994;**19**:163-8.

CONTRIBUTION OF INDIAN SCIENTISTS TO PHARMACOLOGICAL RESEARCH IN THE U.S.A. AND CANADA

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1. Historical perspectives

Science is international. Accordingly, pharmacological research has become a global activity. Due to television and computers, the information is distributed at a faster rate than ever before. Indian heritage in drug research and Ayurvedic medicine has been known for centuries[1,2,3]. Pharmacological science in the USA emerged with the isolation of adrenaline by John J. Abel. Before 1955, most of the Indian scientists in pharmacology were trained in England. About the same time, the hypotensive action of Rauwolfia-serpentina was reconfirmed in the laboratories of The Ohio State University in Columbus, Ohio[4]. The chemical structure of reserpine was elucidated, the unique catecholamine depleting action of the alkaloid was subsequently reported by 1958. A generation of Indian graduate students found their way to the USA and some to Canada for research in pharmacology.

The rational basis of steric structure activity research led to the development of molecular pharmacology and medicinal chemistry[5-8]. Basic knowledge in morphology, chemistry, biochemistry, physiology and pharmaceutical sciences created an attractive platform for the contributions by Indian pharmacologists. In the early seventies, Saradindu Dutta and B.H. Marks, then at The Ohio State University, investigated the mechanism of action of cardiac glycosides and related drugs[9,10]. The work has been well recognized. The group at Ohio continued its interest in natural products and extensive studies were undertaken on the pharmacology of Thalictrum alkaloids. The studies have been reviewed by Patil and Beal[11]. Investigators of Indian origin are contributing to knowledge of drugs at various laboratories in academic institutions, the pharmaceutical industry, private institutions and government agencies. Although attempts are made to organize scientists of Indian descent in various associations, its value for the development of research in pharmacological sciences is slow. The computer library, Internet, as well as biographical books, can easily enhance the understanding of contributions of these scientists.

2. Receptor pharmacology

Optical isomers provided a valuable probe to study structure action relationship for the receptors of classical neurotransmitters, hormones and ions. Sastry[5] investigated a series of asymmetric analogues related to acetylcholine. On the preparations containing muscarinic receptors and nicotinic receptors, an interesting deviation in the ratios of activity of isomers occurred. Like acetylcholine, its analogues are highly flexible around the C-C bond. Thus, the orientation of important functional groups of a series of agonists for the interaction with muscarinic and nicotinic acetylcholine receptors was proposed. The investigators provided insight for the molecular conformations in activation of cholinergic receptors.

Since 1963 to the present, an extensive investigation was launched by Lapidus, Tye and Patil on the sympathomimetic class of substances[8,12]. Validity of the Easson-Steadman hypothesis that the activity of (-)-epinephrine>(+)-epinephrine = desoxyepinephrine was re-investigated. It was postulated that in (-)-epinephrine, three groups attached to asymmetric carbon interact with the active site of the receptor, whereas in (+)-epinephrine, the benzylic OH group may not interact with the receptor, resulting in lesser activity. Desoxyepinephrine, which lacks a benzylic OH group was equiactive with (+)-epinephrine. The hypothesis was found to be true for directly-acting α and β adrenoceptor activating catecholamines and their corresponding desoxy forms. When the theory was extended to some indirectly-acting or catecholamine-releasing substances or the non-catecholamine type of molecules, an interesting deviation occurred. For example, tolazoline, a non-phenolic imidazoline which can be superimposed on clonidine or the α agonist phenylephrine, was more active than its benzylic OH analogues with either R or S conformation. Even catechol derivatives of tolazoline did not confirm the pattern of action expected from the Easson-Steadman hypothesis. The nature of activation of the α-adrenoceptor by classical neurotransmitters and synthetic imidazolines was thus clearly differentiated[13]. These studies on imidazolines were conducted with the close collaboration of Professors D. D. Miller, D. R. Feller and A. Hamada of the divisions of medicinal chemistry and pharmacology.

In another series of experiments on the isolated rat vas deferens where a tissue desensitized to a type of α-agonist oxymetazoline produced contraction to phenylephrine type of α-agonist. The mode of the adrenoceptor activation by two types of agonists must differ[14].

As compared to the neurotransmitter (-)-norepinephrine, many imidazolines are partial agonists. The components of agonist activation originate at the receptor. For the greater efficacy of the agonist, there must be a greater change in the conformation of the receptor. Based on steric structure activity studies, it was postulated that the higher intensity of pharmacologic effects of an agonist like (-)-norepinephrine at the α-adrenoceptor must be due to the sequential interaction of the charged amino group, followed by the catechol group and, finally, the benzylic-OH group must complete the intensity of the conformational change. Alberto Kaumann's work reported that U-5201, the molecule which lacks -NH_2 functionality, activated the β adrenoceptor. Therefore, the sequence of functional groups of (-)-norepinephrine for the activation of the β adrenoceptor may follow the catechol-phenyl group first, benzylic hydroxy and NH_2 may be the last group. It is not that only the receptor protein may differ, but also the mode of activation of the adrenoceptor by a single molecule. Initial stereoselective change in activation of rat lung β adrenoceptor was confirmed by circular dichroism spectroscopy[15].

Opsin, when combined with the vitamin A aldehyde, is referred to as rhodopsin. The seven transmembrane domain G protein coupled-protein shows homology to the β-adrenoceptor. Details of conformational changes in the protein molecule were systematically investigated by Khorana and his colleagues at MIT[16]. When activated by light or dark, the change in conformation of few helixes of the seven transmembrane domains can be independent of each other. There is little or no global conformational change because transmembrane domains are weakly coupled. Thus, there may be little energy expenditure during the activation of this important protein in the visual process. These investigators also proposed a fascinating proton translocation model for bacteriorhodopsin. Unlike that in vertebrate vision, bacteriorhodopsin functions as a proton exchanger from the cytoplasmic side to the extracellular medium of bacteria.

There is a practical utility of stereoisomers of agonists or antagonists for the differentiation of receptors. If receptor types or subtypes differ at the active site, the affinity and/or efficacy ratios of the isomers cannot be equal. Along with other methods, the receptor differentiation based on isomeric activity ratios of pure stereoisomers is still practiced in the characterization of various receptor studies[8,15].

Due to structural simplicity, therapeutic utility and metabolic stability, imidazolines provide important molecular probes for investigation. The evidence for specific functional imidazoline-receptors has been controversial[12]. Imidazoline receptors are characterized as subtypes of the α-adrenoceptor. Chemically reactive SCN-tolazoline was synthesized with the hope that such a reagent would differentiate specific imidazoline receptors from α-adrenoceptors. Smooth or skeletal muscle contraction by the imidazoline at a concentration of approx. 30 μM is not blocked by classical blockers. Only calcium channel blockers and phentolamine at high concentrations block the vascular response. The imidazoline must interact with K_{ATP} and a Ca^{++} channel in muscle. In the rat brain homogenate SCN-tolazoline, however, has been shown to exhibit some specific binding to an unidentified site. Studies based on various types of smooth muscle, including rabbit iris sphincter and dilator, this agent is classified as an "irreversible agonist", *i.e.*

there is initial activation and subsequent desensitization or irreversible block - a unique new category of activity for this molecule[17].

G.N. Pandey and colleagues at Chicago have undertaken in-depth studies on involvement of biogenic amines in pathogenesis of mental disorders and modulation of drug effects. They have demonstrated abnormalities of α_2 adrenergic receptors and the linked adenylate cyclase system in the platelets[18] and decrease in α_2 adrenergic receptor mediated adenylate cyclase system in leucocytes of depressed patients[19]. More recently they have shown an increase in $5HT_{2A}$ receptors in platelets of depressed suicidal patients[20] and also abnormalities in PI signaling system in platelets of depressed patients[21]. Another major finding has been an increase in benzodiazepine receptors and decrease in phospholipase C and protein kinase C in postmortem brain of teenage suicide victims[22,23]. His studies have also elucidated an abnormality of membrane function associated with lithium-sodium counter transport in bipolar illness which may have a genetic control[24]. In acute schizophrenia there is an increase in postsynaptic dopamine receptors[25]. Pandey and colleagues were among the first to show the interaction of antidepressants with β adrenergic receptors and this receptors mediated cyclic AMP response[26] and to suggest that one of the mechanisms of antidepressant therapy may be a decrease in β adrenergic receptors[27].

Parmar and his colleagues have demonstrated the neuromodulatory role of serotonin in mechanisms of action of some anticonvulsant drugs[28,29]. They have also studied cellular mechanisms of disease conditions using high affinity receptor binding of specific drugs and neurotransmitters and shown their presence in human being preparations[30]. The abnormal metabolites of Maple syrup urine disease were found to modulate neurotransmitterr receptor binding[31].

Chandan Prasad and colleagues working at Louisiana State University, New Orleans have demonstrated a new cyclic dipeptide cyclo(His-Pro) in nervous system and endocrines of several mammalian and non-mammalian species and its role in appetite modulation and thermoregulation[32]. They have shown the presence of another endogenous enterostatin-like peptide in the brain and its role in modulation of insulin secretion and appetite[33]. They have discovered a pentapeptide proteoglycan in urine of cachectic patients which causes selective depletion of fat depot and profound weight loss in rodents[34]. They have also demonstrated phospholipid transmethylation as signal transduction mechanism for hormone secretion[35].

Gulati in Chicago has used endothelin agonists and antagonists to elucidate its physiological roles. His studies indicate involvement of sympathetic nervous system in mediation of its central cardiovascular effects[36] and its role in pathogenesis of hypertension[37]. The studies with endothelin antagonists suggest their possible utility in management of cardiovascular disorders[38]. Enodthelin may also play an important role in resuscitative action of hemoglobin therapeutics[36], a diaspirin cross-linked hemoglobin, being developed by them as a blood substitute[39]. Nitric oxide may also be involved in mediatting cardiovascular effects of this product [40,41].

Exogenous dopamine increases blood flow to the kidney in dogs. Goldlberg and Kohli attributed this effect to dopamine receptors, because haloperidol blocked the vasodilatation. Since then, subtypes of dopamine receptors were recognized. Kohli *et al* [42] reported the D_1 agonist activity of dihydrexidine as five times the activity of dopamine. Activity of dihydrexidine at α and β adrenoceptor was absent. Additional dopaminergic mechanisms in kidney were extensively investigated by Lokhandwala and coworkers [43].

Hegde *et al*[44,45] has been actively characterizing the muscarinic receptor of rat urinary bladder, rabbit iris sphincter and dog ciliary muscle. Based on the dissociation constants of anti-muscarinic drugs in functional experiments, and the binding constants *in vivo*, the bladder regulation was attributed to the M_3 subtype of the cholinergic receptor. Furthermore, the role of the M_2 subtype of the receptor, which is negatively coupled to adenelyl cyclase, was also elucidated. The activation of the latter presumably reverses the β adrenoceptor mediated relaxation. Thus, both M_2 and M_3 receptors work synergistically to empty the bladder efficiently. Also, urinary bladder and rabbit iris sphincter contain M_3 subtype of receptor which controls the contraction in both tissues. The co-relation with the human M_5 receptor, if any, was not settled. Furthermore, when the investigators at Roche Bioscience analyzed the contractile responses to (+)-cis dioxolane of the canine ciliary muscle, the muscarinic receptor profile did not fit in the characteristics of M_1 - M_5 subtypes.

Human iris sphincter obtained from the globes of eye bank donors were functionally investigated for the muscarinic receptor subtype. The dissociation constant of atropine either in the sphincter or ciliary muscle was approx 1nM; use of selective blockers did not provide a clear cut profile for a single subtype controlling the contractile function. Multiple receptors in various proportions may control the function[46]. Studies on human iris dilator muscle revealed an interesting aspect of the functional control. First, the maximum mechanical strength of the dilatory muscle is less than 1/3 the contractions by the iris sphincter muscle. Secondly, the α-adrenoceptor exhibits pharmacologic characteristics more like that of the pigmented iris dilator muscle of the *non*-albino rabbit. Thus, the albino rabbit appears to be less useful as a laboratory animal to investigate relevant, therapeutic research[17].

Before chemical and pharmacological characterization of prostaglandins, an extract from iris was named "irin". Recent introduction of an ocular hypotensive prostaglandin analog stimulated more interest in the study of its receptors. Bhattacherjee and Paterson[47] investigated the binding of ligands to ocular tissues of rabbit and bovine membrane. The distribution of ^{3}H-prostaglandin (PGE_2) indicated species differences in the iris sphincter and the ciliary muscle. Investigation should be extended to the human intraocular tissues where drugs act to reduce intraocular pressure.

3. Junctional and intracellular events

At the neuroeffector junction, neuronal and postjunctional cells maintain the functional homeostasis. Alterations in the release of transmitter can alter the sensitivity of the post-junctional receptors. Drugs which affect the disposition of the transmitter also affect the response. It is well known that at the noradrenergic neuroeffector junction cocaine and corticosterone block $uptake_1$ and $uptake_2$, respectively. As a result, response to exogenously administered (-)-norepinephrine is potentiated. The dose response curve of (-)-norepinephrine on the isolated albino rabbit iris-dilator muscle is potentiated by 100 fold[48]. These observations provide remarkable synergism leading to the toxicity in vital organs when two drugs of such nature are combined. On the other hand, regulation of concentration of acetylcholine at the cholinergic junction is markedly influenced by subtypes of acetylcholinesterases. Although Sastry *et al*[49] characterized the esterase type in rat retina, the investigation of that in human tissues remains a challenge.

Activation of the pre- or post-junctional receptors initiates a cascade of cellular events. Some components for activation of the β adrenoceptor include G proteins, adnenyl cyclase, cyclic AMP, AMP and ions. As early as 1974 in our laboratories we observed a stereoselective fat cell lipolysis by stereoisomers of isoproterenol but also stereoselective rise in cAMP. The ratio of EC_{50} of (+)-enantiomer/(-)-enantiomer for the functional event was similar to that for the rise in cAMP[50].

At Wayne State University, Wakade and his colleagues[51-54] conducted an interesting basic investigation on the development and properties of embryonic chick sympathetic neurons in culture. Excess KCl, phorbol 12,13 dibutyrate which cause marked activation of protein kinase C as well as other growth factors were used as tools to investigate the development of the neuron. It was concluded that multiple trophic inputs distinct from the nerve growth factors are required for the normal physiological functions of the noradrenergic neuron. There was remarkable difference in the accumulation of ^{3}H-noradrenaline by intact adrenal medulla of neonatal rat as compared to the 2-6 day old dissociated cells in culture. So-called norepinephrine transporter was 100-1000 times more active in the latter preparation. Glucocorticoid inhibited norepinephrine transporter function of chromaffin cells. Investigators also observed that chronic activation of protein kinase C by phorbol 12, 13-dibutyrate reduces the expression of N-type Ca^{++} channels in the neuron, and 2'-deoxyadenosine selectively kills non-neuronal cells without affecting chick dorsal root ganglia.

In a series of reports, Patel and co-workers at the University of Tennessee investigated the interrelationship between the epidermal growth factor induced accumulation of cAMP. The growth factor increases cAMP only in those cells which express the rat and canine adenyl cyclase V.

Murad, Mittal and co-investigators exhibited remarkable skill and insight by purifying guanylate cyclase, the important regulator of cyclic GMP[55]. Importantly, these investigators demonstrated that nitric oxide or sodium nitroprusside activated the guanylate cyclase. This activation was enhanced up to 40 fold by 1mM hemin, and sodium cyanide in the concentration dependent manner prevented the activation[56]. These original scientific developments formed the basis of the Nobel prize for F. Murad. The story of nitric oxide as well as nitric oxide synthase in relation to the cellular signal transduction by cGMP continues[57,58]. Gopal Krishna and his coleagues at NIH have studied constitutive expression of nitric oxide synthase in human tissues. They have shown that nitric oxide is produced by inducible nitric oxide synthase in human retinal, cerebellar and skeletal muscle tissues. Two types of mRNA were detected in the retina whereas only one type was observed in the other tissues[59]. They have also cloned cDNA sequence of human retinal poly $(A)^+$ RNA and provided further evidence for possible role of nitric oxide in retinal function and neuronal diseases[60].

Receptors and signal transduction mechanisms which control aqueous humor production in the eye have been speculative. Aquaporin, a six-transmembrane domain protein, has been implicated as a water channel receptor. Subtypes of aquaporin receptors in other organs may exist. Vasopressin may control water permeability by activating aquaporin *via* a cAMP-dependent mechanism[61,62].

At Creighton University, Agrawal and a team of investigators reported low and high molecular weight G proteins in tracheal smooth muscle membranes. The role of various G-proteins in relation to disease state must be elucidated[63]. Investigators further observed the rise in second messenger inositol 1, 4,5-triphosphate (IP_3) by platelet-activating factor in U937 cells *via* tyrosine kinase. The rise in the second messenger was augmented in different cells[64]. The literature of the programmed cell death called apoptosis has been reviewed. The methods are carefully described to detect the process at the cellular as well as at the tissue level. The versatile quantitative methods should be useful in understanding the pathophysiology of various diseases[65].

The biochemical cascade resulting from activation of α_1 -adrenoceptor by phenylephrine in fibroblasts has been examined. In adrenoceptor subtype transfected cells, cAMP modulates release of arachidonic acid activated by the agonist. The coupling between α_{1A}, α_{1B} and α_{1D} to phospholipase D and cAMP accumulation has been suggested. The cAMP generated by α_{1A} stimulation acts as inhibitory modulator to phospholipase and arachidonic acid and release *via* protein kinase A [66]. Cellular and intracellular role of various components of signal transduction pathways for vascular angiotensin receptors have been reported[67-69].

Cellular damage caused by free radicals has been known for a long time. *In vitro* hydrogen peroxide incubation decreased with affinity and density of β_1 adrenoceptors. The investigators suggested that hydrogen peroxide may interfere with the functions of Gs proteins and the catalytic subunit of adenyl cyclase enzyme[70]. Increase in hypochlorous acid in ischemic heart has been suggested. It is linked to the inhibition of cardiac sarcolemmal Na^+K^+ATPase activity. The role of oxidative stress on cardiac function is implicated[71].

Inder Verma from the Salk Institute has contributed significantly to our understanding of cellular functions and growth. Investigators report the signal transduction pathways that lead to patterning of vertebrate limb. The Rel/NF-Kappa B family of transcription factors was induced in response to several signals which promote cell growth, differentiation, inflammatory responses, apoptosis and neoplastic transformations. When the activity of Rel/NF-Kappa B protein is blocked by viral vectors that produce trasdominant-negative 1-Kappa/B alpha proteins, limb outgrowth is arrested. The importance of Rel/NF-Kappa B transcription factor in the vertebrate limb development is reported[72].

4. Functional pharmacologic mechanisms

The snyaptic receptors either *in vitro* or *in vivo* are the key in understanding drug action. The quantitative aspects of drug evaluation methods have been reviewed[16].

Sengupta *et al*[73] studied the role of sensory nerves in rat colon. The effect of opioid agonists on the sensory nerves mediated colorectal distension was investigated. Neither morphine, fentanyl nor delta-enkephalin agonist affected responses to colon distension. In contrast, the kappa agonist fedotozine type of drug reduced neuronal responses to the mechanical colon injury. The latter agonist modulates visceral nociception. But kappa-opiod receptors associated with the peripheral sensory nerves appear to be different from those characterized in the brain[74]. A short period of cardiac ischemia can suppress mechano, chemo and ischemia-sensitive activity of cardiac afferent sensory nerves[75]. These neurophysiologic findings are important in relation to drug utility in patients. In the canine sympathetic ganglia, Horn and Kohli[76] verified α_1, α_2 -adrenoceptor and dopamine D_2 receptors. Two inhibitory systems were recognized. One system involved α_1-adrenoceptor and D_2 receptors located proximal to the synapse of the pre- and post-ganglionic neurons and the other α_2 -adrenoceptor located distal to the intraganglionic synapse.

Beta-adrenoceptor which mediates relaxation of bronchial smooth muscle is well known. Pandya found α -adrenoceptor mediating contraction in the trachael smooth muscle of puppies. The receptor disappears in tissues of old dogs[77]. The observation is important.

Swamy, Triggle and co-workers at SUNY Buffalo investigated the role of calcium in vascular activity of drugs[78-81]. They concluded that calcium channel antagonists nifedipine-like drugs were potent inhibitors of both norepinephrine and K^+ induced responses. In the rat portal vein, the inhibitory potency of the antagonist was equal between tissues from control and hypertensive animals. It was also noted that the α -adrenoceptor agonist BHT 920 mainly utilized extracellular calcium, whereas norepinephrine-induced responses mobilized extracellular calcium to varying degrees depending upon the concentration of the agonist used to elicit the contraction. Based on the dissociation constants of nifedipine with the agonist BK 8644, (pA_2 8.3) or with depolarizing concentration of K^+ (pA_2 9.5) there was a 20 fold difference in the affinities of the blocker for the receptor. The state of the channel may differ according to the activating stimuli. Furthermore, dissociation constants of prazosin, yohimbine and corynanthine failed to discriminate pre- and post-junctional α-adrenoceptor in rat tail artery.

Lal *et al* [82] made an interesting observation in relation to the regulation of vascular tone by vitamin B-6, which inhibits influx of Ca^{2+} across the cell membrane. Blood pressure of hypertensive rats can be reduced by the vitamin supplement. There is a chemical similarity between vitamin B-6 and synthetic calcium channel blockers. The observation also forms the basis of the development of synthetic Ca^{2+} channel blockers in the treatment of hypertension.

Chronic administration of many cardiac stimulants produces cardiac hypertrophy. Phosphatidylcholine hydrolysis product, phosphatidic acid, was implicated in the development of the hypertrophy. The hydrolysis product in high concentration may serve as a signal for additional protein synthesis[83]. The role of calcium in cardiac ischemia has been investigated. Adenosine appears important in the calcium homeostasis in the heart[84].

Bhagat has investigated the reduced responsiveness of endotoxin-treated guinea pig atria to norepinephrine and shown that locus of action of the endotoxin may be the heart cell membrane. It may induce certain changes in the sequence of steps involved in excitation-contraction coupling in the myocardium[85]. They demonstrated positive inotropic response to insulin in electrically driven guinea pig atria assoicated with a reduction in norepinephrine response. They explain this sub-sensitivity on the basis of insulin facilitating the uptake of norepinephrine, thereby decreasing the amount reaching the receptors[86].

Due to the complex network of neurons and related transmitters, the functional control of gastrointestinal tract is difficult to elucidate. Rattan and Chakder[87] found insensitivity of anal sphincter responses to atropine, guanethidine, apamin or tetrodotoxin. However, both the contractile and relaxant effects were inhibited by adenylate cyclase-activating peptide antagonist and vasoactive intestinal polypeptide antagonist. The nitric oxide synthase inhibitor reduced the inhibitory but not the excitatory effect of the pituitary cyclase-activating peptide. The latter peptide appears to activate inhibitory receptors on the nerve terminal and excitatory receptors on the internal anal sphincter muscle. The investigators also found nitric oxide synthase mRNA gene expression in neurons and pyloric sphincter muscle[88]. The inhibitory effect of the hemoxygenase antagonist, zinc protopophyrin IX, on the agonists mediated relaxant effect has been investigated[89].

In certain vascular atherosclerotic diseases, there is a leukocyte-endothelial cell interaction which is coordinated by a variety of cell adhesion molecules. Balaram *et al* [90] suggested that insulin-like growth factor-1 may be important in the over expression of cell adhesion molecules leading to greater endothelial cell-monocyte adhesion.

For several years Ticku and his team of investigators at the University of Texas, San Antonio campus, have been studying glutamate and N-methyl-D-asparate (NMDA) receptors in the central nervous system. Activation of these ion channel receptors are important in learning, memory and developmental plasticity. Greater influx of Ca^{++} due to hyperactivation of NMDA receptor can cause neuronal cell death. The mechanism of anticonvulsant effects of losigamone and its optical isomers were investigated in rat brain membranes. Competition of the anticonvulsant with benzodiazepine or picrotoxin binding sites of the GABA receptor was absent. However, losigamone is capable of stimulating chloride influx in spinal chord neurons by a GABA-receptor sensitive allosteric mechanism[91,92]. In cultured cortical cell neurons, chronic neurosteroids treatment promoted the down-regulation of the GABA receptor binding[93]. Restricted caloric diet, as well as chronic alcohol consumption, has modulatory effects on the expression of these receptors[94,95].

Tolerance to morphine, as well as its addiction liabilities, limits clinical utility of the drug in medicine. At The Ohio State University, Tejwani and co-workers reported that the short acting analgesic drug midazolon can produce analgesia and decrease in morphine tolerance and dependence in the rat. Interaction of midazolon with the opioid receptor system was implicated. Morphine tolerant rats exhibited increase in met-enkephalin levels in cortex and midbrain area, but there was a significant decrease in the pituitary and cerebellum. Midazolon appears to inhibit morphine tolerance and dependence by reversing neurochemical changes[96]. Diazepam also inhibits the development of morphine tolerance and dependence in rats. Diazepam treatment antagonizes the up-regulation of μ-opiate receptors observed in morphine tolerant rats[97]. The rise in cAMP during abstinence is also prevented by diazepam[98]. Bhargava reviewed the literature on opioid tolerance and concluded that μ-opioid antagonists block tolerance and dependence on morphine, but these drugs also antagonize the analgesic effect of morphine. Delta-selective opioid antagonists do not modify morphine analgesia[99]. In a series of papers, Bhargava included the role of inhibitor of nNOS and NMDA receptors in the chronic effects of morphine[100,101].

5. Drug delivery, gene therapy and animal models

Bioavailability of many topical ophthalmic medications is less than 1%. The cornea presents a major barrier for drug transport. In humans, Corneal Barrier Index for the mydriatic effect of phenylephrine may be as high as 40,000. Very small amounts are transported to the intraocular site of action[102]. Topical medications, however, may produce high plasma concentration that may cause adverse changes in vital organs like heart and bronchioles. Kumar *et al* [103] reported about gels as ophthalmic delivery systems that can be prepared by a combination of carbopol and methyl cellulose by rational cone and plate viscometry. Two drugs containing liquids can shift the gel phase in the eye. The idea needs to be implemented. A new ion exchange resin was reported for delivery of the antiglaucoma medication betaxolol. The formulation increased the bioavailability of the drug significantly with increased ocular comfort[104]. Drug-containing polymonic particles that are erodible, or ion-exchange resins suspended in a liquid carrier medium, are called micro-particulates. When instilled in the eye, the drug is slowly released from microparticulates. The new formulations hold a great promise for drug delivery[105].

Delivery of genes to cells remains a major problem in therapeutics. Verma and his group administered a recombinant viral vector, which carried instructions to express the canine factor IX protein in transgenic mice[106]. The publication was highly cited by scientists who are investigating gene therapy. The investigators constructed a new series of lentivirus vectors based on HIV-1 that can transduce non-dividing cells. They have used the vector to deliver genes directly into liver and muscle and obtained sustained expression[107]. Injection of viruses into the sub-retinal space of the rat eye showed that wild-type vector transduced predominantly retinal pigment epithelium and photoreceptor cells, while SIN vector was able to transduce other types of retinal cells. The latter SIN HIV vectors should be safer for gene therapy[108,109].

With the tools and techniques in molecular biology, it is possible to obtain specific receptor or enzyme deficient animal models. Wang *et al* [110] produced factor IX-deficient mice for gene therapy of Hemophilia, B. In rabbits, intra vitrial *E-coli* endotoxin causes major breakdown of the blood-aqueous barrier and neutrophils infiltrate into aqueous humor. Administration of dexamethasone and other immunosuppressant drugs inhibit the toxin-induced uveitis[111,112]. The model is useful in eye research. Some years ago, G. Krishna and co-investigators at NIH developed difficult biochemical assays for studying physiologic, pharmacologic and toxicological research. Their important contribution is recognized.

Chronic alcoholics exhibit withdrawal symptoms, which are difficult to treat. In animal models, the monosialoganglioside reduces ethanol mediated acute intoxication and symptoms associated with its withdrawal[113]. Subhash Pande and his colleagues at Chicago have demonstrated the involvement of serotonin receptors in alcoholic dependence and withdrawal[114]. Pharmacokinetics of mutamycin C was studied with and without embolilzation in dogs. Gelfoam does not provide sustained local release of mutamycin C as compared to intravenous infusion. Selective renal mutamycin C infusion without an effective embolic agent does not reduce systemic levels compared with intravenous therapy[115]. In a canine model of infrarenal aortic cross-clamping, vasoactive peptides are released following ischemia. The model is useful to investigate the phenomenon of angiogenesis[116]. Pigs are popular in diet-induced arteriosclerosis research. Many pathological changes in the swine model are similar to those in humans[117].

Hirschsprung's disease in humans is characterized by megacolon. A mouse model of the disease has been developed to investigate the pathogenesis of the inhibitory transmission and receptors of the distal colon and anal sphincter[118].

Wallis and Lal[113] report that the discriminative stimuli produced by piperazine analog are based upon its selective actions on serotonin receptors. This animal model should be a useful tool to investigate serotonergic anxiolytic drugs.

Canine vein preservation in different storage media at lower temperature and extended times produced some morphological changes in the endothelial and vascular smooth muscle. The relationship of these morphological changes to graft failure was co-related. Investigators recommend the storage medium of the University of Wisconsin solution superior to the normal saline or autologous whole blood[119].

Innervation of intraocular blood vessels is questionable. The vascular tone mainly depends on the local hormonal and ionic changes. Kulkarni and Payne[120] described a bovine retinal microcirculation, which responds to prostastaglandins and related substances. The preparation is useful in ocular drug research[121].

Giri *et al* [122] reported a model of lung fibrosis and decorin that may provide a useful substance in treating pulmonary fibrosis observed in patients.

Hasan Mukhtar and colleagues at Case Western Reserve University, Cleveland have been working skin pharmacology and carcinogenesis utilising murine and human skin. They have demonstrated multiple inducible forms of cytochrome P450 in the skin and that 2 members of this multigene family of enzymes, CYP1A1 and CYP1B1 are predominantly expressed following exposure to UV radiation or chemical carcinogens[123]. They have shown that polyphenolic fraction isolated from green tea affords protection against all stages of cutaneous carcinogenesis[124], solar erythema in human skin[125] and also against collagen-induced arthritis in mice[126]. He has demonstrated that photodynamic therapy (PDT) holds promise for treatment of various dermatological disorders like psoriasis, alopecia areata and certain skin cancers[127].

6. Pharmacokinetics in neonates, children and patients with cystic fibrosis

In 1999, Professor Milap C. Nahata, The Ohio State University, was the recipient of the American Pharmaceutical Associations research achievement award in the pharmaceutical sciences. With physicians and students, Dr. Nahata conducted a remarkable clinical research at Childrens Hospital in Columbus. A summary of his contributions is as follows: They evaluated pharmacokinetics of dexamethasone in 9 premature neonates with a mean gestational age of 27 weeks and a postnatal age of 22 days. Although there was a considerable variation from one subject to another, neonates less than 27 weeks gestation had almost a five-fold lower clearance of dexamethasone than those greater than 27 weeks gestation. The drug half-life ranged from 2-16 hours. These parameters are useful in pharmacotherapy of dexamethasone. Systolic and diastolic blood pressure were significantly elevated during the early course of the therapy[128]. They have also investigated pharmacokinetics of phenytoin in children with neurotrauma[129]. Ear infection causes significant problems in children. Efficacy of topical ciprofloxacin in 29 children, average 5 years, was investigated. Nearly 70% of patients with otorrhoea associated with *P. aeruginosa* were effectively cured without adverse reactions[130].

African-American children 1.5 to 11 years of age were treated for *Trichophyton tonsurans* tinea capitis. The anti-fungal agent itraconazole, at a dose of 100 mg/day for 4 weeks, was effective in less than half the children treated. The dose and duration of the treatment may improve the successful treatment of the infection in the majority of children[131]. Respiratory tract infection in 18 patients, average age 25 years, was treated with tobramycin infusion 7-15 mg/kg. The mean dose of 12 mg/kg was needed to obtain optimum serum concentration of the antibiotic. A single maintenance dose reduces the potential ototoxicity of the repeated daily doses of the antibiotic[132]. There is also a cost savings associated with the relevant therapy.

7. Side effects and chemical toxicity

Drug-induced adverse effects have been documented. Risk benefit ratio for a particular medication is constantly monitored under the careful watch of the Food and Drug Administration, as well as the Environmental Protection Agency. Several scientists of Indian heritage are working in these institutions. Environmental toxicity- causing chemicals may interfere with normal physiological functions or drug-induced effects. Study of these toxicity mechanisms is important so that the course of drug therapy can be altered, specific antagonists or protective agents can be developed or toxicants can be removed from the environment.

During the last few years, a prostaglandin analog latanoprost has been introduced for the treatment of open angle glaucoma. Patients receiving topical medication developed unilateral or bilateral uveitis after chronic use of the drug. The anterior uveitis improved after cessation of latanoprost with or without topical corticosteroids. Topical use of such medication may be contraindicated in patients with a history of uveitis or prior ocular surgery[133].

Many liposoluble drugs are bound to the melanin pigment in various organs including eyes, hair and inner ear[134]. The drugs or chemicals which are bound to the pigment may produce local toxicity.

At the Southern Illinois University, a group of investigators studied the protection of cisplatin-induced ototoxicity in rats by 4-methylthiobenzoic acid [135]. Cisplatin treatment in animals markedly reduced superoxide dismutase, glutathione reductase, and related enzymes. 4-Methylthiobenzoic acid and cisplatin treated rats did not show significant elevation of hearing threshold, an indicator of the protective effect. The protective agent may do so by restoring the cochlear antioxidant system. Somani and co-investigators continue their work with antioxidant system activity and changes in different types of acetylcholine esterases for understanding the chronic effects of ethanol[136-138]. Other equally important global issues of acute and chronic effects of pesticide residues in the air, water and foods are being investigated by many scientists.

Molecular mechanisms in the development of lens opacities of various types are being studied. In age-related cataract, internal and external chemical insults compound the problem. At the University of Maryland, Varma and his colleagues are investigating the protective role of ascorbate, taurine, pyruvate and its esters on the biochemical damage of the vertebrate lens. The research provides rational molecular mechanisms in understanding cataract formation and its prevention[139-142].

The age-related impairment of cognitive and motor functions associated with oxidative molecular damage was reported in mice[143]. Various behavioral tests were used. The investigators reached an interesting conclusion that age-related declines of cognitive and motor performance progress independently, and involve oxidative molecular damage with different regions of the brain.

For several years Desaiah *et al* have been studying biochemical changes induced by toxicants in mammalian systems. Neurotoxicity by carbon tetrachloride, cardiac effects of phencyclidine, and disease mediated by other toxicants were reported[144,145].

In the health care sector, the drugs, chemicals or environmental factors which produce embryonic toxicity will continue to demand attention of many. Mehendale and his team[146] at the College of Pharmacy and Health Sciences, Northeast Louisiana University, are elucidating the biochemical mechanisms associated with development of toxicity by pollutants and other toxic chemicals. Parkhie *et al*[147] reported teratogenicity of dimethoxyethyl phthalate in rats. Reports like these demand attention of chemical companies where workers or the surrounding community may be affected by these substances.

REFERENCES

1. Anand N. Contribution of Ayurvedic medicine to medicinal chemistry. In Comprehensive Medicinal Chemistry; vol. 1 (Chair. Ed. Board C. Hansch) New York, Pergamon Press, 1990, pp 113-31.
2. Sanyal PK. A story of medicine and pharmacy in India (second edition) Published by A. Sanyal, Columbus, Ohio USA 1992.
3. Kaul PN. Drug discovery - past, present and future. *Prog Drug Res* 1998;**50**:9-105.
4. Nelson JW, Schlagel CA. A preliminary note on a pharmacological investigation of the hypotensive action of *Rauwolfia serpentina* Benth. *J Amer Pharm Assoc* 1953;**42**: 324.
5. Sastry BVR. Steroisomerism, drug action in the nervous system. *Ann Rev Pharmacol* 1973;**13**:253-67.
6. Patil PN, LaPidus JB, Tye A. Steric aspects of adrenergic drugs. *J Pharm Sci* 1970;**59**:1205-34.
7. Patil PN, LaPidus JB. Stereoisomerism in adrenergic drugs. *Rev Physiol* 1972;**66**:213-60.
8. Patil PN, Miller DD, Trendelenburg UG. Molecular geometry and adrenergic drug activity. *Pharmacol Rev* 1974; **26**:323-92.
9. Dutta S, Marks BH. Species and ionic influences on the accumulation of digitalis glycosides by isolated perfused hearts. *Brit J Pharmacol* 1972;**46**:401-8.
10. Ito M, Hollander PB, Marks BH, Dutta S. The effects of six cardiac glycosides on the transmembrane potential and contractile characteristics of the right ventricle of guinea pig. *J Pharmacol Exp Ther* 1970;**172**:188-95.
11. Patil PN, Beal JB. Acitivities of Thalictrum alkaloids; *Trends Pharmacol Sci* 1987;**8**:327-9.
12. Patil PN. Investigation of pharmacologic receptors for catecholamines and imidazolines. *The Auto N System* (*J Japan Soc Neuroveg Res*) 1998;**35**:29-35.
13. Patil PN, Feller DR, Miller DD. Molecular aspects of the receptor activation by imidazolines: an overview. *J Neurol Trans (Suppl)* 1991;**34**:187-94.
14. Ruffolo RR, Turowiski B, Patil PN. Lack of cross tachyphylaxis between structurally dissimilar α-adrenoceptor agonists. *J Pharm Pharmacol* 1977 ;**29**:378-80.
15. Patil PN. Pharmacologic quantitation. *Indian J Exptl Biol* 1996;**34**:615-33.
16. Kim JM, Altenbach C, Thurmond RL, Khorana HG, Hubell WL. Structure and function of rhodopsin: rhodopsin mutants with a neutral amino acid at E134 have a partially activated conformation in the dark. *Proc National Acad Scics U.S.A.* 1997 ;**94**:1427-8.
17. Ishikawa H, Miller DD, Patil PN. Irreversible agonist and antagonist properties of isothiocyanatobenzyl imidazoline in albino rabbit iris muscles. *N S Arch Pharmacol* 1996;**354**: 362-8.
18. Wang YC, Pandey GN, Mendels J , Frazer A. Platelet adenylate cyclase responses in depression: Implications for a receptor defect. *Psychopharmcologia* 1974;**36**: 291-300.
19. Pandey GN, Janicak P, Davis JM. Decreased beta-adrenergic receptors in the leucocytes of depressed patients. *Psychiat Res* 1987;**22**:265-73.
20. Pandey GN, Pandey SC, Janicak PG, Marks RC, Davis JM. Platelet serotonin-2 receptor binding sites in depression and suicide. *Biol Psychiat* 1990;**22**:265-73.
21. Dwivedi Y, Janicak PG, Pandey GN. Elevated [3H] inosital 1,4,5-triphosphates receptor protein level in platelets of depressed patients. *Psychopharmacol* 1998;**138**:47.
22. Pandey GN, Conley RR, Pandey SC, Goel S, Roberts RC *et al*. Benzodiazepine receptors in the postmortem brain of suicide victims and schizophrenic subjects. *Psychiatry Res* 1997;**71**:137-49.
23. Pandey GN, Dwivedi Y, Pandey SC, Conely RR, Roberts RC, Tamminga CA. Protein kinase C in the postmortem brain of teenage suicide victims. *Neurosc Lett* 1997;**228**: 111-4.
24. Pandey GN, Dorus E, Davis JM, Tosteson DC. Lithium transport in human red blood cells. Genetic and clinical aspects. *Arch Gen Psychiat* 1979;**36**:902-8.
25. Pandey GN, Ostrow DG, Haas M, Dorus E, Casper RC *et al*. Abnormal lithium and sodium transport in erythrocytes of a maniac patient and members of his family. *Proc*

Nat Acad Scics U.S.A. 1977;**74**:3607-11.

26. Frazer A, Pandey GN, Mendels J, Neeley S, Kane M, Hesse ME. The effect of tri-iodothyronine in combination with imipramine on [3H] cyclic AMP production in slices of rat cerebral cortex. *Neuropharmacol* 1974;**13**:1131-40.

27. Pandey GN, Davis JM. Treatment with antidepressants, sensitivity of beta adrenergic receptors and affective illness. In: Neuroreceptors Basic and Clinical Aspects eds E Usdin, W E Bunney and J M Davis New York: John Wiley and Sons; 1981.pp 99-120.

28. Leadbetter LM, Brumleve SJ, Parmar SS. The neurotransmitter involvement of serotonin as a possible mediator of the anticonvulsant activity of benzodiazepines. *Res Com Psychol Psychiat Behav* 1985;9:423-4.

29. Leadbetter LM, Parmar SS. Serotonin as a facilitator neurotransmitter in the anticonvulsant activity of methaqualone. *Physiol Behaviour* 1989;**46**:105-6.

30. Parmar SS, Barthwal JP, Seth PK, James EJ. High affinity neurotransmitter and drug receptors in human being preparations. *Proc Western Pharmacol Soc* 1988;**31**:209-13.

31. Dwivedi C, James EC, Parmar SS. Effects of abnormal metabolites of Maple syrup urine disease on neurotransmitter receptor binding. *Biochem Med Metabolic Biol* 1986;**35**:275-8.

32. Prasad C. Bioactive cyclic dipeptides. *Peptides* 1995;**16**: 151-64.

33. Mizuma H, Makoto I, Tiemann WE, Prasad C. Biology of Enterostatin II. Development of enzyme-linked immunosorbent assay (ELISA) for enterostatin (Val Pro-Asp-Pro-Arg), the procolipse activation peptide. *Biochem Biophys Res Commun* 1995;**215**:227-34.

34. Figueroa J, Vijayagopal P, Prasad A, Schapira DV, Prasad C. Isolation, characterization and distribution of a 29KD proteoglycan in the urine of cachetic cancer and aids patients. *Biochem Biophys Res Commun* 1999;**254**:642-6.

35. Prasad C, Edwards RM. Synthesis of phosphatidylchotine from phosphatidylethanolamine by at least two methyltransferases in rat pituitary extracts. *J Biol Chem* 1981; **256**:13000-3.

36. Gulati A, Kumar A, Morrison S, Shahani BT. Effect of centrally administered endothelin agonists on systemic and regional blood circulation in the rat/role of sympathetic nervous system. *Neuropeptides* 1997;**31**:301-9.

37. Gulati A, Artwohl JE, Kumar A, Gao XP, Rubinstein I. Endothelin-1 like immunoreactivity in a new rodent model of spontaneous hypertension. *Amer J Hyperten* 1998;**11**: 866-9.

38. Kumar A, Morrison S, Gulati A. Effect of ET-A receptor antagonists on cardiovascular responses induced by centrally administered sarafatoxin 6b/role of sympathetic nervous system. *Peptides* 1997;**18**:855-64.

39. Kumar A, Sen AP, Saxena PR, Gulati A. Resuscilation with diaspirin crosslinked hemoglobin increases cerebral and renal blood perfusion in hemorrhaged rats. *Artificial Cells Blood Substitutes Immob Biotechnol* 1997;**25**:85-94.

40. Sen A P, Dong Y M, Saxena PR, Gulati A. Modulation of resuscitative effect of diaspirin cross-linked hemoglobin by L-NAME in rats. *Shock* 1998;**9**:223-30.

41. Sharma AC, Singh G, Gulati A. Role of NO mechanism in cardiovascular effects of diaspirin cross-linked hemoglobin in anaesthetised rats. *Amer J Physiol - Heart & Circulat Physiol* 1995;**38**:H1379-H88.

42. Kohli JD, Horn PT, Glock D, Brewster WK, Nichols DE. Dihydrexidine: a new potent peripheral dopamine D_1 receptor agonist. *Europ J Pharmacol* 1993;**235**:31-5.

43. Hussain T, Lokhandwala MF. Renal dopamine receptor function in hypertension. *Hypertension* 1998;**32**:187-97.

44. Hegde SS, Choppin A, Bonhaus D, Briaud S, Loeb M *et al.* Functional role of M_2 and M_3 muscarinic receptors in the urinary bladder of rats *in vitro* and *in vivo. Brit J Pharmacol* 1997;**120**:1408-9.

45. Hegde SS, Eglen RM, Loury D, Choppin A. Do M_3 muscarinic receptors mediate contraction of dog ciliary smooth muscle? Studies with darifenacin; N.-S. Arch. Pharmacol. Abstract, XIII Int. Congress, Munich, Germany. 1998.

46. Ishikawa H, DeSantis L, Patil PN. Selectivity of muscarinic agonists including (+)-aceclidine and antimuscarinics on the human intraocular muscles. *J Ocul Pharmacol* 1998; **14**:363-73.

47. Bhattacherjee P, Paterson CA. Studies on prostanoid receptors in ocular tissues. *J Ocular Pharmacol* 1994;**10**:167-75.

48. Ishikawa H, Miller DD, Patil PN. Comparison of postjunctional α-adrenoceptors in iris dilatory muscle of humans and albino and pigmented rabbits. *N S Arch Pharmacol* 1996;**154**:765-72.

49. Sastry BVR, Kambam SR, Sing G, Franks JJ. Nature of cholinesterase in the rat retina. *J Ocul Pharmacol* 1994; **10**:195-201.

50. DeSantis L, Feller DR, Patil PN. Drug receptor related stereoselectivities in fat cells. *Europ J Pharmacol* 1974; **28**:302-9.

51. Przywara DA, Kulkarni JS, Wakade TD, Wakade AR.

Importance of protein kinase C for normal development of transmitter release properties in embryonic chick sympathetic neuron in culture. *Neuroscience* 1996;**72**:815-20.

52. Przywara DA, Zong H, Kulkarni JS, Anderson GF, Wakade AR. Chronic activation of protein kinase C by phorbol ester reduces calcium channel expression in chick sympathetic neurons. *Neurosc Lett* 1997;**229**:138-40.

53. Wakade AR, Wakade TD, Poosch M, Bannon MJ. Noradrenaline transport and transporter mRNA of rat chromaffin cells are controlled by dexemethasome and nerve growth factor. *J Physiol* 1996;**494**:67-75.

54. Wakade AR, Kulkarni JS, Fuji JT. 2'-Deoxyadenosine selectively kills nonneuronal cells without affecting survival and growth of chick dorsal root ganglion. *Brain Res* 1998; **788**:69-79.

55. Murad F, Lewicki JA, Brandwein H, Mittal CK, Waldman A. Guanylate cyclase purification, free radical activation, radiolabelling and preparation of hydridoma antibodies. *Adv Cyclic Nucl Res* 1981;**14**:229-39.

56. Lewicki JA, Brandwein HJ, Mittal CK, Arnold WP, Murad F. Properties of purified soluble guanylate cyclase activated by nitric oxide and sodium nitroprusside. *J Cycl Nucl Res* 1982;**8**:17-25.

57. Mittal CK. Nitric oxide synthase involvement of oxygen radicals in conversion of L-arginine to nitric oxide. *Biochem Biophys Res Comm* 1993;**193**:126-32.

58. Mittal CK. Oxygen-radical/nitric oxide mediate calcium-dependent action on cyclic GMP system: a novel concept in signal transduction mechanisms. *Mol Cellu Biochem* 1995;**149-150**:257-62.

59. Park CS, Park R, Krishna G. Constitutive expression and structural diversity of inducible isoform of nitric synthase in human tissues. *Life Sciences* 1996;**59**:219-25.

60. Park CS, Gianotti C, Park R, Krishna G. Neuronal isoform of nitric oxide synthase is expressed at low levels in human retina. *Cellu Mol Neurobiol* 1996;**16**:499-515.

61. Patil RV, Han Z, Wax MB. Regulation of water channel activity of aquaporin by arginine vasopressin and atrial natriuretic peptide. *Biochem Biophys Res Comm* 1997;**238**: 392-6.

62. Patil RV, Saito I, Yang Y, Wax MB. Expression of aquaporins in the rat ocular tissue. *Exptl Eye Res* 1997;**64**:203-9.

63. Joshi S, Abebe W, Agrawal DK. Identification of guanine nucleotide binding regulatory protein in bovine tracheal smooth muscle. *Mol Cellu Biochem* 1996;**154**:179-84.

64. Abebe W, Ali N, Agrawal DK. Platelet-activating factor-induced inositol 1,4,5-triphosphate generation in undifferentiated, differentiated U937 cells. *Int J Immunopharmacol* 1996;**18**:173-81.

65. Allen RT, Hunter WJ, Agrawal DK. Morphological and biochemical characterization and analysis of apoptosis. *J Pharmacol Toxicol Methods* 1997;**37**:215-28.

66. Ruan Y, Kan H, Parmentier JH, Fatima S, Allen L, Malik KU. Alpha-1 adrenergic receptor stimulation with phenylephrine promotes arachidonic acid release by activation of phospholipase D in rat-1 fibroblasts: inhibition by protein kinase A. *J Pharmacol Exp Ther* 1998;**284**:576-85.

67. Muthalif MM, Benter IF, Karzoun N, Fatima S, Harper J, Uddin MR, Malik KU. 20-Hydroxyeicosate traenoic acid mediates calcium/calmodulin-dependent protein kinase II induced nitrogen-activated protein kinase activation in vascular smooth muscle. *Proc Nat Acad Scics U.S.A.* 1998;**95**: 1270-6.

68. Muthalif MM, Benter IF, Uddin MR, Harper H, Malik KU. Signal transduction mechanisms involved in angiotensin (1-7)-stimulated arachidonic and release and prostanoid synthesis in rabbit aortic muscle cells. *J Pharmacol Exp Ther* 1998;**284**:388-98.

69. Jagadeesh G. Angiotensin II receptors - Antagonists, molecular biology and signal transduction. *Ind J Exptl Biol* 1998;**36**:1171-94.

70. Persad S, Rupp H, Jindal H, Arneja J, Dhalla NS. Modification of cardiac beta-adrenoceptor mechanisms by H_2O_2. *Amer J Physiol* 1998;**274**:H416-23.

71. Kato K, Shao O, Elimban V, Lukas A, Dhalla NS. Mechanism of depression in cardiac sarcolemmal Na^+K^+ATPase hypochlorous acid. *Amer J Physiol* 1998;**275**:C826-31.

72. Kanegae Y, Tavares AT, Izpisua-Belmonte JC, Verma IM. Role of Rel/NF-kappa B transcription factor during the outgrowth of the vertebrate limb. *Nature* 1998;**392**:611-4.

73. Sengupta JN, Su X, Gebhart GF. Kappa but not mu or delta, opioids attenuate responses to distention of afferent fibers innervating the rat colon. *Gastroenterol* 1996;**11**: 968-70.

74. Su X, Sengupta JN, Gebhart GFG. Effects of kappa opioid receptor-selective agonists-selective agonists on responses of pelvic nerves afferents to noxious coloretal distension. *J Neurophysiol* 1997;**78**:1003-12.

75. Abe T, Morgan D, Sengupta JN, Gebhart, Gutterman DD. Attenuation of ischemia-induced activation of cardiac sympathetic afferents following brief myocardial ischemia in rats. *J Aut N System* 1998;**71**:28-36.

76. Horn PT, Kohli JD. Studies on the location of catecholamines receptors in canine sympathetic ganglia. *Europ J Pharmacol* 1992;**224**:103-7.

77. Pandya K. Postnatal developmental changes in adrenergic receptor responses of the dog tracheal muscle. *Arch Int Pharmacodyn Ther* 1977;**230**:53-64.

78. Harris AL, Swamy VC, Triggle DJ. Calcium reactivity and antagonism in portal veins from spontaneously hypertensive and normotensive rats. *Can J Physiol Pharmacol* 1984; **62**:146-50.

79. Rutledge A, Swamy VC, Triggle DJ. Calcium-dependence and antagonism of responses to alpha-1 and alpha-2-adrenoceptor agonist in vascular tissue from hypertensive and normotensive rats. *Brit J Pharmacol* 1984;**83**:103-11.

80. Su CM, Swamy VC, Triggle DL. Calcium channel activation in vascular smooth muscle by BAY, K8644. *Can J Physiol Pharmacol* 1984;**62**:1401-10.

81. Su CM, Swamy VC, Triggle DL. Postsynaptic alpha-adrenoceptor characterization and Ca^{2+} channel antagonist and activator action in rat tail arteries from normotensive and hypertensive animals. *Can J Physiol Pharmacol* 1986;**64**:901-21.

82. Lal KJ, Sharma SK, Dakshinamurti K. Regulation of calcium influx into vascular smooth muscle by vitamin B-6. *Clin Exp Hyperten* 1993;**15**:489-500.

83. Dhalla NS, Xu YJ, Sheu SS, Tappia PS, Panagia V. Phosphatidic acid: a potential signal transducer for cardiac hypertrophy. *J Mol Cellu Cardio* 1997;**29**:2865-71.

84. Kawabata K, Osada M, Netticadan T, Dhalla NS. Beneficial effects of ischemic preconditioning on Ca^{2+} paradox in the heart. *Life Sci* 1998;**63**:685-92.

85. Bhagat B, Rao PS, Cavanagh D. Contractility of endotoxic atria. *Adv Myocardiol* 1980;**2**:199-207.

86. Bhagat B, Burke WJ, Dhalla NS. Insulin-induced enhancement of uptake of noradrenaline in atrial strips. *Brit J Pharmacol* 1981;**74**:325-32.

87. Rattan S, Chakder S. Excitatory and inhibitory actions of pituitary adenylate cyclase-activating peptide (PACAP) in the internal sphincter smooth muscle sites of action. *J Pharmacol Exp Ther* 1997;**283**:722-8.

88. Chakder S, Bandyopadhyay A, Rattan S. Neuronal NOS gene expression in gastrointestinal myenteric neurons and smooth muscle cells. *Amer J Physiol* 1997;**273**:C1868-75.

89. Fan VP, Chakder S, Rattan S. Inhibitory effects of zinc protoporphyrin IX, on lower esophageal sphincter smooth muscle relaxation by vasoactive intestinal polypeptide and other receptor agonists. *J Pharmacol Expt Ther* 1998;**285**: 468-75.

90. Balaram SK; Agrawal DK, Allen RT, Kuszynski CA, Edwards JD. Cell adhesion molecules and insulin-like growth factor-1 in vascular disease. *J Vascul Surg* 1997;**25**:866-76.

91. Robinson R, Ticku MR. N-methyl-D-asparate (NMDA) receptor: friend or foe? *Ind J Exptl Biol* 1998;**36**:947-59.

92. Dimpfel W, Chatterjee SS, Noldner M, Ticku MK. Effects of the anticonvulsant losigamone and its isomers on the GABA receptor system. *Epilepsia* 1995;**36**:983-89.

93. Yu R, Follesa P, Ticku MK. Down-regulation of the GABA receptor subunits mRNA levels in mammalian cultured cortical neurons following chronic neurosteroid treatment. *Brain Res Mol Brain Res* 1996;**41**:163-8.

94. Mhatre MC, Ticku MK. Caloric restriction retards the aging associated changes in gamma-aminobutyric acid receptor gene expression in rat cerebellum. *Brain Res Mol Brain Res* 1998;**54**:270-5.

95. Kalluri HS, Mehta A, Ticku MK. Up-regulation of NMDA receptor subunits in rat brain following chronic ethanol treatment. *Brain Res Mol Brain Res* 1998;**58**:221-4.

96. Tejwani GA, Rattan AK. Met-enkephalin alteration in the rat during chronic injection of morphine and/or midazolam. *Brain Res* 1997;**775**:119-26.

97. Tejwani GA, Sheu MJ, Shribanditmongkol P, Satyapria A. Inhibition of morphine tolerance and dependence by diazepam and its relationship to μ-opioid receptors in the rat brain and spinal cord. *Brain Res* 1998;**797**:305-12.

98. Sheu MJ, Shribanditmongkol P, Santosa DN, Tejwani GA. Inhibition of morphine tolerance and dependence by diazepam and its relationship to cyclic AMP levels in discreet rat brain regions and spinal cord. *Brain Res* 1995; **675**:31-7.

99. Bhargava HN. Drugs that modify opioid tolerance, physical dependence and abstinence symptoms: Preclinical and clinical studies. *NIDA Res Monograph* 1995;**147**:53-83.

100. Bhargava HN, Cao YJ, Zhao GM. Effects of 7-nitroindazole on tolerance to morphine, U-50, 488H and [D-Pen-2, D-Pen-5] enkephalin in mice. *Peptides* 1997;**18**:797-800.

101. Bhargava HN, Sharma SS, Bian JT. Evidence for a role of N-methyl-D-aspartate receptors in L-arginine-induced attenuation of morphine antinociception. *Brain Res* 1998;**782**: 314-7.

102. Patil PN, Ishikawa H. Corneal barrier index in ocular pharmacotherapeutics; Abstract, 31st Indian Pharmacology Soc. Meeting, Lucknow, India, Dec. 18-20, 1998.

103. Kumar S, Haglund B, Himmelstein KJ. In situ-forming gels

for ophthalmic drug delivery. *J Ocul Pharmacol* 1994;**10**:47-56.

104. Jani R, Gan O, Ali Y, Rodstrom R, Hancock S. Ion exchange resins for ophthalmic delivery. *J Ocul Pharmacol* 1994; **10**:57-67.

105. Joshi A. Microparticulates for ophthalmic drug delivery. *J Ocul Pharmacol* 1994;**10**:29-45.

106. Dai Y, Schwartz E, Gu D, Zhang W, Sarvetnick N, Verma IM. Cellular and humoral responses to adenoviral vectors containing factor IX gene. Tolerization of factor IX and vector antigens for long term expression. *Proc Nat Acad Sci U.S.A.* 1995;**92**:1401-5.

107. Kafri T, Blomer U, Peterson DS, Gage FH, Verma IM. Sustained expression of genes delivered directly into liver and muscle by lentiviral vectors. *Nature Genetics* 1997;**17**:314-7.

108. Miyoshi H, Takahashi M, Gage F, Verma IM. Stable and efficient gene transfer into the retina using an HIV-based lentiviral vector. *Proc Nat Acad Scics U.S.A.* 1997;**94**: 10319-23.

109. Miyoshi H, Blomer U, Takahashi M, Gage F, Verma IM. Development of a self inactivating lentivirus vector. *J Virol* 1998;**72**:8150-7.

110. Wang L, Zoppe M, Hackeng T, Griffin J, Lee K , Verma IM. A factor IX deficient mouse model for hemophillia B gene therapy. *Proc Nat Acad Scics U.S.A.* 1997;**94**:11563-6.

111. Ohia E, Mancino M, Kulkarni PS. Effects of steroids and immunosuppressive drugs on endotoxin-uveitis in rabbits. *J Ocular Pharmacol* 1992;**8**:295-307.

112. Kulkarni PS. Steroidal and non-steroidal drugs in endotoxin-induced uveitis. *J Ocul Pharmacol* 1994;**10**:329-34.

113. Wallis CJ, Lal H. A discriminative stimulus produced by 1-(3-cholorophenyl)-piperazine (mCPP) as a putative animal model of anxiety. *Neuro-Psycho-Pharmacol Biol Psych* 1998;**22**:547-65.

114. Pandey SC, Dubey MP, Piano MR, Schwertz DW, Davis JM, Pandey GN. Modulation of $5HT_{1C}$ receptors and phosphoinositide system by ethanol consumption in rat brain and choroid plexus. *Europ J Pharmacol* (*Mol Pharmacol Section*) 1993;**247**:81-8.

115. Zhan X, Thorpe P, Agrawal DK, Leo H, Yan X. Pharmacokinetic, angiographic and histologic comparison of catheter-directed chemoembolization versus systemic chemotherapy in a canine model. *Can J Physiol Pharmacol* 1996; **74**:1117-25.

116. Dovgan PS, Edwards JD, Rowley JM, Agrawal DK, Adrian T. Effects of ischemia and reperfusion on vasoactive neuropeptide levels in the canine infra-renal aortic revascularization model. *Cardiovasc Surg* 1996;**4**:470-5.

117. Thorpe PE, Hunter WJ, Zhan X, Dovagan P, Agrawal DK. A noninjury diet-induced swine model of arteriosclerosis for cardiovascular-interventional research. *Angiol* 1996; **47**:849-57.

118. Chakder S, McHugh K, Rattan S. Inhibitory neurotransmission in lethal spotted mutant mice, a model for Hirschsprung's disease. *Gastroenterol* 1997;**112**:1575-85.

119. Cavallari N, Abebe W, Mingoli A, Hunter WJ, Agrawal DK, Cavallaro A, Edwards J. Functional and morphological evaluation of canine veins following presentation in different storage media. *J Surg Res* 1997;**68**:106-15.

120. Kulkarni P, Payne S. Eicosanoids in bovine retinal microcirculation. *J Ocul Pharmacol Ther* 1997;**13**:139-49.

121. Kulkarni P, Joshua IG, Roberts A, Barnes G. A novel method to assess reactivities of retinal microcirculation. *Microvasc Res* 1994;**48**:39-49.

122. Giri SN, Hyde DM, Braun RK, Gaarde W, Harper JR, Pierschbacher MD. Antifibrotic effect of decorin in a bleomycin Hamster model of lung fibrosis. *Biochem Pharmacol* 1997;**54**:1205-16.

123. Elemets CA, Athar M, Tubesing KA, Rothaupt D, Xu H, Mukhtar H. Susceptibility to the biological effects of polyaromatic hydrocarbons is influenced by genes of the major histocompatibility complex. *Proc Natl Acad Scics U.S.A.* 1998;**95**:14915-9 .

124. Ahmad N, Feyes DK, Nieminen AL, Agarwal R, Mukhtar H. Green tea constituent epigallocatechin 3-gallate and induction of apoptosis and cell cycle arrest in human carcinogma cells. *J Natl Cancer Inst* 1997;**89**:1881-6.

125. Katiyar SK, Matsui MS, Elmets CA, Mukhtar H. Polyphenolic antioxidant (-) epigallocatechin-3-gallate from green tea reduces UVB-induced inflammatory responses and infiltration of leukocytes in human skin. *Photochem Photobiol* 1999;**69**:148-53.

126. Haqqui TM, Feyes DK, Agarwal R, Mukhtar H. Prevention of collagen-induced arthritis in mice by polyphenolic antioxidants from green tea. *Proc Natl Acad Sci U.S.A.* 1999; **96**:4524-9.

127. Ahmad N, Feyes DK, Agarwal R, Mukhtar H. Photodynamic therapy results in induction of WAF1/C1P1/P21, leading to cell cycle arrest and apoptosis. *Proc Natl Acad Sci U.S.A.* 1998;**95**:6977-82.

128. Lugo R, Nahata MC, Menke JA, McLead Jr., RE. Pharmacokinetics of dexamethasone in premature

neonates . *Europ J Clin Pharmacol* 1996;**49**:477-83.

129. Bahal O'Mara N, Richard Jones P, Anglin D, Cox S, Nahata MC. Pharmacokinetics of phenytoin in children with neurotrauma. *Crit Care Med* 1995;**23**:1418-24.

130. Wintermeyer SM, Hart MC, Nahata MC. Efficacy of ototopical ciprofloxacin in pediatric patients with otorrhea. *Otolaryn Head Neck Surg* 1997;**116**:450-3.

131. Abdel-Rahman SM, Powell DA, Nahata MC. Efficacy of itraconazole in children with *Trichophyton-tonsurans* tinea capitis. *J Amer Acad Dermatol* 1998;**38**:44-6.

132. Bates RD, Nahata MC, Jones JW, McCoy K, Young G, Cox S, Barson WJ. Pharmacokinetics and safety of tobramycin after once-daily administration in patients with cystic fibrosis. *Chest* 1997;**112**:1208-13.

133. Fechtner RD, Khouri AS, Zimmerman TJ, Bullock J, Feldman R, Kulkarni P, Michael A, Realini T, Warwar R. Anterior uveitis associated with latanoprost. *Amer J Ophthalmol* 1998;**126**:37-41.

134. Salazar-Bookaman M, Wainer I, Patil PN. Relevance of drug-melanin interactions to ocular pharmacology and toxicology. *J Ocul Pharmacol* 1994;**10**:217-39.

135. Rybak L, Hussain K, Evenson L, Morris C, Whitworth C, Somani SM. Protection by 4-methylthiobenzoic acid against cisplatin-induced ototoxicity-antioxidant system. *Pharmacol Toxicol* 1997;**81**:173-9.

136. Somani SM, Hussain K. Interaction of exercise training and chronic ethanol ingestion on antioxidant system of rat brain regions. *J Appl Tox* 1997;**17**:329-36.

137. Hussain K, Somani SM. Influence of exercise and ethanol on cholinesterase activity and lipid peroxidation in blood and brain regions of rat. *Prog Neuro Psycho Pharmacol Biol Psych* 1997;**21**:659-70.

138. Hussain K, Morris C. Whitworth C, Trammell GL, Rybak LP, Somani SM. Protection by ebselen against cisplatin-induced neurotoxicity antioxidant system. *Mol Cell Biochem* 1998;**178**:127-33.

139. Devamanoharan PS, Morris SM, Verma SD. Oxyradical scavenging effects of dehydroascorbate. *Mol Cellu Biochem* 1996;**156**:131-4.

140. Varma SD, Ali AH, Devamanoharan PS, Morris SM. Nitrite induced photo oxidation of thiol and its implications in smog toxicity to the eye: Prevention by ascorbate. *J Ocul Pharmacol Ther* 1997;**13**:179-87.

141. Devamanoharan PS, Ali A, Verma SD. Prevention of lens protein glycation by taurine. *Mol Cellu Biochem* 1997;**177**: 245-50.

142. Varma SD, Devamanoharan PS, Ali A. Prevention of intraocular oxidative stress to lens by pyruvate and its ester. *Free Rad Res* 1998;**28**:131-5.

143. Forster MJ, Dubey A, Dawson K, Stutts WA, Lal H, Sohal RS. Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain. *Proc Nat Acad Sci U.S.A.* 1996;**93**:4765-9.

144. Pande M, Cameron JA, Vig PJ, Desaiah D. Phencyclidine block of Ca^{2+} ATPage in rat heart sarcoplasmic reticulum. *Toxicol* 1998;**129**:95-102.

145. Pentyala SN, Vig PJ, Sekhon BS, Desaiah D. Effects of carbon tetrachloride on inositol 1,4,5-triphosphate dependent and independent regulation of rat brain microsomal Ca^{2+} flux. *Cellu Signl* 1994;**6**:561-7.

146. Dalu A, Rao PS, Mehendale HM. Colchicine antimitosis abolishes resiliency of postnatally developing rats to chlordecone-amplified carbon tetrachloride hepatoxicity and lethality. *Environ Health Perspect* 1998;**106**:597-606.

147. Parkhie MR, Webb M, Norcross MA. Dimethoxy phenyl phthalate: embryopathy, teratogenecity, fetal metabolism and the role of zinc in the rat. *Environ Health Perspec* 1982; **45**:89-97.